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(54) Title: HYBRID GROWTH FACTORS

## (57) Abstract

The present invention provides recombinant hematopoietic molecules comprising at least a portion of a first hematopoietic molecule having early myeloid differentiation activity and at least a portion of a second hematopoietic molecule having late myeloid differentiation activity. Nucleic acid molecules encoding such recombinant molecules, as well as pharmaceutical compositions comprising such recombinant factors are also disclosed.

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### HYBRID GROWTH FACTORS

#### BACKGROUND OF THE INVENTION

5 Within this application several publications are referenced by Arabic numerals within parentheses. Full citations for these references may be found at the end of the specification immediately preceding the Sequence Listing. The disclosures of these publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this 10 invention pertains.

A variety of factors can influence the activity of a cell. Frequently 15 a factor exerts its influence by interacting with a receptor on the surface of a cell. After binding to the receptor, the signal which determines the cellular response to the factor can be mediated through a number of different events, including internalization of the factor or alterations of the receptor caused by ligand binding. During the 20 course of hematopoietic differentiation, a number of different factors are involved in the maturation of a pluripotent stem cell into a fully differentiated cell. The activities of these factors during the course of hematopoietic differentiation have resulted in these factors being characterized as early factors or late factors. For example, 25 factors such as interleukin-3 (IL-3) and granulocyte-macrophage colony stimulating factor (GM-CSF) are considered early factors, while erythropoietin (Epo), macrophage colony stimulating factor (M-CSF), and granulocyte colony stimulating factor (G-CSF) are considered late factors.

30 Based upon studies performed with purified factors and in vitro colony forming unit assays, it appears that both IL-3 and GM-CSF act on pluripotent cells before they become committed to a particular hematopoietic pathway. After the events stimulated by these factors are underway, such lineage restricted cells become receptive to 35 further differentiation mediated by such late factors as Epo, (which leads to the maturation of erythrocytes), G-CSF (which leads cells

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into the granulocytic pathway), and M-CSF (which leads to the maturation of macrophages). Experiments described in recent publications (1,2,3) have demonstrated in vitro that early or late factors alone are poor stimuli of colony formation. However, when an 5 early factor such as IL-3 or GM-CSF is combined with a late factor, levels of colony formation equivalent to that seen with conditioned media having full activity is observed. Thus, differentiation appears to be dependent upon the dual activities of early and late factors.

10 Despite a clear requirement for both IL-3 or GM-CSF and Epo for the formation of erythroid colony forming units, published results indicate that IL-3 can down-modulate high affinity Epo receptors (4). Because the amount of IL-3 required to demonstrate down-modulation of 15 the Epo receptor was higher than that reported by others who demonstrated functional full IL-3 activity in the presence of Epo, it is unclear whether this phenomenon is relevant in vivo.

20 Previous experiments in animals (22-26) suggest that under conditions of hematopoietic regeneration, optimal expansion of late progenitors could only occur in the presence of an adequate early progenitor pool. This then makes manipulations that result in the expansion of early 25 hematopoietic progenitor pools extremely desirable. IL-3 has been shown to exert a differentiative and proliferative effect on early progenitor cells and at IL-3 concentrations which had little or no effect alone, Epo acted synergistically to induce proliferation and differentiation of erythroid progenitors. (27) By targeting a molecule with both early (IL-3) and late (Epo and G-CSF) activities to early progenitor cells, optimal expansion of a desired lineage should be possible.

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SUMMARY OF THE INVENTION

The present invention concerns hybrid molecules comprising early and late differentiation factors produced by genetic manipulation. By covalently linking such factors the local concentration of the late factor is very high at the surface of a cell to which the early factor is bound. Additionally, if down-modulation is relevant *in vivo*, binding of late factors to any remaining low-affinity receptors, e.g. Epo receptors, could be enhanced, thus reducing the amount of late factor required to stimulate the cell. Furthermore, by linking an early factor with a late factor, such early factor may act more specifically to stimulate only the desired lineage, thus reducing any undesirable effects mediated by the early factor. Finally, it is considerably easier to produce and administer to a patient a single factor with two activities rather it would to produce and administer two separate factors.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a Western blot analysis of IL-3/Epo hybrid growth factors in CHO CM. CHO CM was collected from clones 23-10 (IL-3:Epo Flex), 5-4 (IL-3:Epo Short) and 17-3-1 (Epo:IL-3 Short). Hybrid growth factor concentrations were determined by ELISA assay. CM containing 74 ng of IL-3:Epo Flex (having a 23 aa flexible linker (lane 2), 73.5 ng of IL-3:Epo Short (having a short 2 aa linker) (lane 3), 80 ng of Epo:IL-3 Short (having a 3 aa linker) (lane 4) were subjected to SDS-PAGE (10-20% gel) electrophoresis and were assayed for Epo by Western blotting with a mouse anti-Epo polyclonal antisera as described in Example 7. Medium conditioned by CHO cells transfected with the vector pEe6 (lane 5) and rHu Epo 10 ng (lane 6), 20 ng (lane 7), 30 ng (lane 8), 70 ng (lane 9), and 100 ng (lane 10) were included. Molecular size markers in kilodaltons (lane 1).

Figure 2 shows AML193 cells proliferate in response to the IL-3 moiety of the hybrid growth factors. AML193 cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS and growth

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factor deprived for 16 hours. The indicated concentrations of growth factors were added for 42 hours followed by a 6 hour pulse of (<sup>3</sup>H) thymidine as described in Example 7. NO GF (no growth factor); CHO CM (medium conditioned by CHO cells transfected with the vector pEe6); 5 Epo (rHu Epo); IL-3 (rHu IL-3); IL-3:Epo Flex (CHO CM containing IL-3:Epo fusion protein with a 23aa flexible linker); IL-3:Epo Short (CHO CM containing IL-3:Epo fusion protein with a 2aa linker); Epo:IL-3 Short (CHO CM containing Epo:IL-3 fusion protein with a 3aa linker).

10 Figure 3 shows dose response of IL-3 adapted AML193 cells to the IL-3 moiety of the hybrid growth factors. IL-3 adapted AML 193 cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS minus growth factor for 16 hours. Increasing concentrations of IL-3 and fusion proteins were added and the assay was carried out as 15 described in Figure 2 and in Example 7. IL-3:Epo Flex (CHO CM containing IL-3:Epo fusion protein with a 23aa flexible linker); IL-3:Epo Short (CHO CM containing IL-3:Epo fusion protein with a 2aa linker); Epo:IL-3 Short (CHO CM containing Epo:IL-3 fusion protein with a 3aa linker).

20 Figure 4 shows FDC-P1/ER cells proliferate in response to the Epo moiety of the hybrid growth factors. FDC-P1/ER cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS without growth factor for 16 hours. The indicated concentrations of growth 25 factors were added for 42 hours followed by a 6 hour pulse of (<sup>3</sup>H) thymidine as described in Example 7. Columns are labeled as described in Figure 2. WEHI3 CM (medium conditioned by murine WEHI3 cells which produce and secrete IL-3).

30 Figure 5 shows dose response of FDC-P1/ER cells to the Epo moiety of the hybrid growth factors. FDC-P1/ER cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS and deprived of growth factor for 16 hours. Increasing concentrations of Epo and fusion proteins were added and the assay was carried out as described in 35 Example 7. Hybrid growth factors are as designated in Figure 3.

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Figure 6 shows IL-3 plus Epo responsiveness of IL-3 adapted TF-1 cells. TF-1 cells adapted for growth in IL-3 were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS minus growth factor for 16 hours. Increasing concentrations of growth factors, 0.75 fmol/ml hybrid growth factors and 0.75 fmol/ml Epo plus 1.5 fmol/ml IL-3 (1), 1.5 fmol/ml hybrid growth factors and 1.5 fmol/ml of Epo plus 3.0 fmol/ml IL-3 (2), 3.0 fmol/ml hybrid growth factors and 3.0 fmol/ml Epo plus 6.0 fmol/ml IL-3 (3), were added and the assay was carried out as described in Example 7. Hybrid growth factors are as designated in Figure 3.

Figure 7 shows dose responsiveness of IL-3 adapted TF-1 cells to the hybrid growth factors. TF-1 cells adapted for growth in IL-3 were grown to log phase and suspended in RPMI-1640 plus 10% FCS minus growth factor for 16 hours. Increasing concentrations of hybrid growth factors were added and the cells were incubated for 8 hours. (<sup>3</sup>H) Thymidine (1  $\mu$ Ci/well) was added and the incubation was continued for 16 hours. (A) Dose response to hybrid growth factor, concentrations of 0 to 30 fmol/ml. (B) Represents the same data as in A for concentrations of 0 to 1.875 fmol/ml to emphasize the differences between hybrid factors. Hybrid growth factors are as designated in Figure 3.

Figure 8 shows dose responsiveness of GM-CSF adapted TF-1 cells to the hybrid growth factors. TF-1 cells maintained in GM-CSF were grown to log phase and suspended in RPMI-1640 plus 10% FCS minus growth factor for 16 hours. Increasing concentrations of hybrid growth factors were added and the assay was carried out as described above for Figure 5. (A) Dose response to hybrid growth factor concentrations, of 0 to 30 fmol/ml. (B) Represents the same data as in A for concentrations of 0 to 1.875 fmol/ml to emphasize the differences between hybrid factors. Hybrid growth factors are as designated in Figure 3.

Figure 9 shows TF-1 cells proliferate in response to the IL-3 moiety of the IL-3/G-CSF hybrid growth factor. TF-1 cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS deprived of

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growth factor for 16 hours. The indicated concentrations of growth factors were added and the assay was carried out as described in Example 7. Factors are as designated in Figure 2 except, G-CSF (rHu G-CSF); IL-3/G-CSF (CHO CM containing IL-3/G-CSF fusion protein with a 10aa linker).

Figure 10 shows NFS-60 cells proliferate in response to the G-CSF moiety of the IL-3/G-CSF hybrid growth factor. NFS-60 cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS minus growth factor for 16 hours. The indicated concentrations of growth factors were added and the assay was carried out as described in Example 7. Growth factors are as designated in Figures 2 and 9.

Figure 11 shows dose responsiveness of AML193 cells to the IL-3:G-CSF hybrid growth factor. AML193 cells were grown to stationary phase and suspended in RPMI-1640 plus 10% FCS deprived of growth factor for 16 hours. The indicated concentrations of growth factors were added and the assay was carried out as described in Example 7. Growth factors are as designated in Figures 2 and 9.

20

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a recombinant hematopoietic molecule comprising at least a portion of a first hematopoietic molecule having early myeloid differentiation activity and at least a portion of a second hematopoietic molecule having late myeloid differentiation activity. This recombinant molecule has early myeloid differentiation activity associated with the first hematopoietic molecule and late myeloid differentiation activity associated with the second hematopoietic molecule. Within this application, "hematopoietic molecule" means a molecule which promotes and/or regulates hematopoiesis. Hematopoietic molecules exert such promotional or regulatory activities at different stages during hematopoiesis, such stages being referred to herein as early myeloid differentiation and late myeloid differentiation. Also within this application, "early myeloid differentiation activity" means the ability to promote the

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differentiation, self-renewal, or proliferation of pluripotent myeloid cells, i.e., stem cells or colony forming unit, granulocyte-erythrocyte-monocyte-megakaryocyte, cells. Moreover, within this application, "late myeloid differentiation activity" means the ability to promote the maturation or differentiation of a lineage restricted myeloid cell, i.e., a myeloid precursor cell committed to a specific cell lineage such as erythrocytes, megakaryocytes, monocytes, neutrophils, eosinophils, and basophils.

5

10 In one embodiment of the invention, the first hematopoietic molecule is selected from the group consisting of IL-3 and GM-CSF. In another embodiment of the invention, the second hemopoietic molecule is selected from the group consisting of Epo, G-CSF, IL-5 and M-CSF. In a preferred embodiment of the invention, the portion of the first hematopoietic molecule is linked to the portion of the second hematopoietic molecule by an amino acid linker sequence comprising at 15 least two amino acid residues.

20 Within the context of the present invention, it is understood that variations in proteins and nucleic acids exist among individuals, e.g. amino acid or nucleotide substitutions, deletions, insertions, and degree or location of glycosylation, and that functional derivatives resulting therefrom are included within the scope of the present invention.

25

30 In a preferred embodiment of the invention, the recombinant molecule comprises the entire amino acid sequence of human IL-3 (SEQ ID NO: 1). Moreover, the recombinant hematopoietic molecule may preferably comprise a 79 amino acid sequence derived from human IL-3 (SEQ ID NO: 2), i.e. residues 1-79 of SEQ ID NO: 1.

Further still, in yet another preferred embodiment of the invention, the recombinant molecule comprises the entire amino acid sequence of human erythropoietin (SEQ ID NO: 3). In still a further embodiment of 35 the invention, the hemopoietic molecule comprises a 155 amino acid

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sequence derived from human erythropoietin (SEQ ID NO: 4), i.e., residues 7-161 of SEQ ID NO: 3.

5 In another preferred embodiment of the invention, the recombinant hematopoietic molecule comprises the entire amino acid sequence of human G-CSF (SEQ ID NO: 5).

10 In one embodiment of the invention, the first hematopoietic molecule is IL-3 and the second hematopoietic molecule is erythropoietin. The first hematopoietic molecule, i.e. IL-3, may comprise the amino portion and the second hematopoietic molecule, i.e. Epo, may comprise the carboxyl portion of the recombinant molecule. Preferably, the recombinant hematopoietic molecule comprises the amino acid sequence from amino acid 1 to amino acid 302 of SEQ ID NO: 6. Also preferably, 15 the recombinant hematopoietic molecule comprises the amino acid sequence from amino acid 1 to amino acid 321 of SEQ ID NO: 7. However, in another embodiment of the invention, the first hematopoietic molecule, i.e. IL-3, may comprise the carboxyl portion and the second hematopoietic molecule, i.e. Epo, may comprise the amino portion of the recombinant molecule. In a preferred embodiment of the 20 invention, the recombinant molecule comprises the amino acid sequence from amino acid 1 to amino acid 303 of SEQ ID NO: 8. In yet another preferred embodiment, the recombinant molecule comprises the amino acid sequence from amino acid 1 to amino acid 322 of SEQ ID NO: 9.

25 In still a further embodiment of the invention, the first hematopoietic molecule is IL-3 and the second hematopoietic molecule is G-CSF. In one such embodiment, the first hematopoietic molecule comprises the amino portion and the second hematopoietic molecule comprises the carboxyl portion of the recombinant molecule. In yet a 30 more specific embodiment, the recombinant molecule comprises the amino acid sequence from amino acid 1 to amino acid 317 of SEQ ID NO: 10.

35 The subject invention also provides nucleic acid molecules which encode the recombinant hematopoietic molecules of the subject invention. Examples of such nucleic acid molecules are SEQ ID NO: 11,

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SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15. Moreover, vectors which comprise the nucleic acid molecules of the subject invention are also disclosed. In one embodiment of the invention, the vector comprises a plasmid. Moreover, host vector systems for the production of a recombinant hematopoietic molecule of the present invention are provided which comprise a vector of the present invention in a suitable host, preferably a mammalian cell such as a CHO or COS cell. This host vector system may be grown under suitable conditions which permit the expression of the recombinant hematopoietic molecule, which may be recovered by purification techniques known in the art, e.g. ion exchange chromatography, affinity chromatography, and size exclusion chromatography.

The present invention further provides pharmaceutical compositions useful for treating patients suffering from anemias of various origins, e.g. renal failure, and AIDS. Moreover, these pharmaceutical compositions are useful for administering to patients for preoperative autologous blood donations, patients receiving or donating bone marrow for transplantation purposes, and patients undergoing cancer chemotherapy. These pharmaceutical compositions comprise effective hematopoiesis-promoting amounts of a recombinant molecule of the present invention and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are known in the art and are disclosed in The Pharmacopeia of the United States and the National Formulary. Depending on the specific application contemplated, the pharmaceutical composition may be formulated as a solution, suspension, parenteral preparation, or spray. Parenteral preparations may include a vehicle such as specially distilled, pyrogen-free water, phosphate buffer, or normal saline. Oral and/or transmucosal dosage forms may comprise phospholipids, often in the form of liposomes.

Also provided is a method for treating a patient to promote hematopoiesis which comprises administering to the patient an effective hematopoiesis-promoting amount of a pharmaceutical composition of the present invention.

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The recombinant hematopoietic molecules, nucleic acid molecules, pharmaceutical compositions and methods of the present invention will be better understood by reference to the following experiments and examples, which are provided for purposes of illustration and are not to be construed as in any way limiting the scope of the invention, which is defined by the claims appended hereto.

### Examples

10 Construction of the hybrid protein genes: Genes encoding IL-3 (SEQ ID NO: 16), Epo (SEQ ID NO: 17) and G-CSF (SEQ ID NO: 18) were purchased from British Biotech. Ltd. These genes were utilized to construct three different hybrid hematopoietic proteins, i.e., IL-3:Epo, Epo:IL-3 and IL-3:G-CSF. In these hybrids the first named gene forms the amino portion and the second named gene the carboxyl portion of the hybrid protein.

#### Example 1

20 A nucleic acid molecule encoding an IL-3:Epo hybrid growth factor was constructed as follows: CSF, the native leader sequence of IL-3 was synthesized as 4 oligonucleotides (SEQ ID NOS: 19-22; see Table I) which represents both strands of the leader sequence. In addition, the 5' end of the leader (SEQ ID NO: 19) encoded a convenient restriction enzyme overhang (EcoR1), although the EcoR1 site was not regenerated, in front of the ATG start codon. The 3' end of the leader (SEQ ID NO: 21) included the first several amino acid codons of IL-3 and an SpeI overhang so that the annealed leader sequence could be easily ligated to IL-3, which was altered by British Biotech to include an SpeI site. The leader sequence was annealed and ligated to pKS (Stratagene Cloning Systems, Inc., San Diego, CA) cleaved with EcoR1 and SpeI. The resulting plasmid was designated pKS0. The IL-3 containing pUC18 plasmid obtained from British Biotech was cleaved with SpeI and NheI, then ligated to a linker oligonucleotide (complimentary oligonucleotide SEQ ID NOS: 23 and 24; see Table I) which contained the following three restriction sites: NheI, XbaI and

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NcoI. Cleavage was then performed with SpeI and XbaI. The resulting 379 base pair fragment was then ligated to PKSO cleaved with 5 SpeI and XbaI. The resulting plasmid (pKS0IL-a) contained the IL-3 leader, the IL-3 gene and a small linker fragment.

The Epo gene was inserted into pEe6 (Celltech, Ltd., Slough, U.K.), a mammalian expression vector which contains the human Cytomegalovirus promoter, a polylinker region and a poly-A addition site in addition 10 to ampicillin resistance and a bacterial origin of replication, by cleaving the Epo containing plasmid obtained from British Biotech with HindIII and BamH1. Epo was then cleaved with NcoI. The same linker comprising oligonucleotide SEQ ID NOS: 23 and 24 as described earlier was ligated to Epo and then cleaved with XbaI to yield the entire Epo 15 gene. This was then ligated to XbaI and BclI cleaved pEe6 to yield pEe6 containing the Epo gene (pEepo). PKS0IL-a was cleaved with EcoRV and an XbaI linker was ligated to the blunt ends followed by cleavage with XbaI, which released the IL-3 gene with the leader sequence. This was then ligated to XbaI cleaved Peepo to yield a plasmid 20 containing an entire hybrid protein gene (pEepie-a) (see SEQ ID NO: 11 for the structure of the inserted hybrid gene, designated herein IL-3:Epo Short). The glutamine synthetase (gs) gene was then inserted into the BamH1 site of pEepie-a to yield pEepogs-a or pEpgs-b, depending upon the orientation of the gs gene. Glutamine synthetase 25 confers resistance to methionine sulphoximine (MSX) in order to select cells which have taken up the plasmid after transfection. After the plasmid was constructed a large batch was grown, purified by CsCl ultracentrifugation, and used for transfection. At each step in this process all ligation joints between fragments were analyzed by DNA 30 sequence analysis in order to assure that there were no changes that would cause frameshifts and prevent the hybrid gene from being expressed.

To construct a nucleic acid molecule encoding an IL-3:Epo hybrid 35 growth factor with a longer linker sequence separating IL-3 and Epo, pEepie-a was cleaved with NheI and annealed oligonucleotide SEQ ID

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NOS: 25 and 26 (see Table I) were ligated into the cleaved plasmid. This linker encodes the flexible amino acid sequence Gly Ser Gly Ser Gly Ser (SEQ ID NO: 27). Clones with the insert in the proper orientation were selected by probing colonies with the junction oligonucleotide SEQ ID NO: 28 (see SEQ ID NO: 14 for the structure of the inserted hybrid gene, designated herein IL-3:Epo Flex). The glutamine synthetase gene was then added to the construct as described above.

10 Example 2

A nucleic acid molecule encoding an IL-3:G-CSF hybrid growth factor was constructed as follows: pUC18 containing G-CSF (British Biotech) was cleaved with HindIII. A linker composed of an overhanging XbaI site, a NotI site and an overhanging HindIII site (oligonucleotide SEQ ID NOS: 29 and 30; see Table I) was ligated to the pUC18:G-CSF. This was then cleaved with XbaI and BamHI which released the entire G-CSF gene. The G-CSF fragment was then inserted into XbaI and BclI cleaved pEe6 (pEe6:G-CSF). IL-3 with its signal sequence was removed from the IL-3:Epo plasmid pEepogs-a as an XbaI fragment. This IL-3 fragment was then inserted into XbaI cleaved pEe6-G-CSF. After restriction analysis, a plasmid containing the IL-3 gene in the proper orientation was obtained (pEG11), this plasmid encoded a gene capable of expressing IL-3 and G-CSF as a hybrid protein (see SEQ ID NO: 13 for the structure of the inserted hybrid gene, designated herein IL-3:G-CSF). The gs gene was inserted into this plasmid as described in Example 1 above to yield plasmids pEG13 and pEG14, depending upon the orientation of the gs gene.

30 Example 3

A nucleic acid molecule encoding an Epo:IL-3 hybrid growth factor was constructed by first synthesizing the native Epo signal sequence as oligonucleotide SEQ ID NOS: 31-36 (see Table I). These were annealed to yield an overhanging 5' XhoI sequence and a 3' PstI sequence. These were then ligated and subcloned as an XhoI/PstI fragment

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(pEpol). In order to obtain the proper reading frame and signal sequence processing site, the plasmid containing the signal sequence was cleaved with PstI and the 3' overhang left by PstI was enzymatically removed with T4 polymerase. This was then cleaved with 5 BamH1. The Epo gene was then amplified by PCR as a fragment with a 5' blunt end using oligonucleotide SEQ ID NO: 37 as a primer and a 3' BamH1 end using oligonucleotide SEQ ID NO: 38 as a primer. This fragment was then ligated into pEpol to yield a complete Epo gene with 10 its leader sequence. PCR was used to amplify the Epo gene with its signal sequence as an (5') XbaI and (3') NotI fragment using oligonucleotide SEQ ID NOS: 39 and 40 as primers. This was then digested with XbaI and NotI. At the same time, a purified IL-3 fragment was amplified by PCR as a (5') NotI and (3') BamH1 fragment using oligonucleotide SEQ ID NOS: 41 and 42, followed by digestion 15 with NotI and BamH1. These two fragments were ligated to pEe6 cleaved with XbaI and BclI to yield a full length hybrid gene encoding both Epo and IL-3 (pEG16) (see SEQ ID NO: 12 for the structure of the inserted hybrid gene, designated herein Epo:IL-3 Short). The gs gene was inserted as described in Example 1 above to yield pEG17 and pEG18, 20 depending upon the orientation of the gs gene.

A flexible linker is inserted into Epo:IL-3 by cleaving pEG17 or pEG18 with NotI. Annealed oligonucleotide SEQ ID NOS: 43 and 44 are then ligated into the cleaved plasmid. Clones with the insert in the proper orientation are selected by probing colonies with a junction 25 oligonucleotide as described above (see SEQ ID NO: 15 for the structure of the inserted hybrid gene.)

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TABLE I  
OLIGONUCLEOTIDES

All oligonucleotides are listed in the 5' to 3' orientation:

5      AATTGCCGCC ACCATGAGCC GCCTGCCGT CCTGCTCCT (SEQ ID NO: 19)  
GCTCCAACTC CTGGTCCGCC CCGGACTCCA AGCTCCCATG ACCCAGACAA (SEQ ID NO: 20)  
CTAGTTGTCT GGGTCATGGG AGCTTGGAGT CCGGGGCGG (SEQ ID NO: 21)  
ACCAGGAGTT GGAGCAGGAG CAGGACGGGC AGGCAGGCTCAT GGTGGCGGC (SEQ ID NO: 22)  
CTAGCGATCT TTCTAGA (SEQ ID NO: 23)  
10     CATGTCTAGA AAGATCG (SEQ ID NO: 24)  
CTAGAACCGG CCGCA (SEQ ID NO: 29)  
TTCGCCGGCG TTCGA (SEQ ID NO: 30)  
TCGAGGCCATG GGGGTGCACG AATGTCCT (SEQ ID NO: 31)  
GCCTGGCTGT GGCTTCTCCT GTCCCTGCTG TC (SEQ ID NO: 32)  
15     GCTCCCTCTG GGCCTCCCAG TCCTGGGCTG CA (SEQ ID NO: 33)  
GCCCAAGGACT GGGAGGCCA GAGGGA (SEQ ID NO: 34)  
GCGACAGCAG GGACAGGAGA AGCCACAGCC AGGCAGGACA TT (SEQ ID NO: 35)  
CGTGCACCCC CATGGC (SEQ ID NO: 36)  
GCCCCACAC GCCTCATCTG T (SEQ ID NO: 37)  
20     GAATTCCGGAT CCTTATCATC T (SEQ ID NO: 38)  
CTAGTCTCTA GAATGGGGGT CCACGAATGT (SEQ ID NO: 39)  
AGCCATGGCG GCCGCTCTGT CCCCTGTCCCT (SEQ ID NO: 40)  
GACAGAGCGG CCGCCATGGC TCCCATGACC (SEQ ID NO: 41)  
GAATTCCGGAT CCTTACTAAA AGATCGCTAG (SEQ ID NO: 42)  
25     CTAGCGTCCG GAGGCAGGTGG CTCGGGCGGT GGCGGCTCGG GTGGCGGCC GCTCTGCG  
(SEQ ID NO: 25)  
CTAGCGCAGA GCCGCCGCCA CCGCAGCCGC CACCGCCCGA GCCACCGCC TCCGGACG  
(SEQ ID NO: 26)  
TTGTCGCTAG CGTCCGGAGG C (SEQ ID NO: 28)  
30     GGCCGCTTCC GGAGGCAGGTG GCTCGGGCGG TGGCGGCTCG GGTGGCGGC GGCTCTGC  
(SEQ ID NO: 43)  
GGCCGCGAGAG CCGCCGCCAC CCGAGCCGCC ACCGCCCCAG CCACCGCCT CCGGCAGC  
(SEQ ID NO: 44)

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Example 4

Transfection of the hybrid gene containing plasmids. All transfections were performed using the Lipofectin<sup>®</sup> transfection kit (Bethesda Research Labs, Gaithersburg, MD) using 15-30 mg. of purified plasmid DNA (pEepogs-a, pEepogs-b, pEG13, pEG14, pEG17, and pEG18). The following alterations were made to the protocol provided by the company: the growth medium in these experiments was GMEM-S and the CHO-K1 cells were incubated in the presence of 10% CO<sub>2</sub>; after addition of the Lipofectin:DNA complex, cells were incubated without selection for 24 hours. The cells were transferred to GMEM-S supplemented with 25 mM MSX after 24 hours. The MSX concentration was subsequently increased to 50 mM after one week. Cloning rings were used to subclone MSX resistant colonies and each of these colonies was placed into an individual well of a 24 well plate. Selected clones were incubated in the absence of MSX to insure that the hybrid protein gene was stably integrated. Strongly positive clones were grown in large cultures to provide larger amounts of hybrid proteins for further analysis.

20

Example 5

Assays for hybrid protein production. Cell supernatants from transfected or control cells were assayed using several different assays. In order to demonstrate Epo production, an RIA kit for Epo was used (Incstar Corp., Stillwater, MN). The presence of IL-3 was determined using an ELISA assay in which the capture antibody was a polyclonal goat anti-IL-3 (R&D Systems, Minneapolis, MN) and the probe antibody was a murine anti-IL-3 monoclonal. Goat anti-mouse conjugated to horseradish peroxidase followed by suitable substrate was used to detect the presence of the monoclonal anti-IL-3. A very similar assay was used to demonstrate the presence of the hybrid proteins except that a murine anti-Epo monoclonal or anti-G-CSF monoclonal was used in place of anti-IL-3 monoclonal. Additionally, IL-3:Epo Short was analyzed by Western blot analysis. The blot was probed with antibody to Epo and then with <sup>125</sup>I goat anti-mouse. A

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single broad band appeared on the autoradiogram with a molecular weight of slightly more than 50,000 daltons.

Example 6

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Cellular assays. Epo and/or IL-3 dependent and responsive cell lines were used to test the biological activities of the hybrid proteins. B6SUTA (5) is a multipotential hematopoietic progenitor cell line established from nonadherent cell populations removed from continuous 10 B6.S mouse bone marrow culture. This cell line demonstrates absolute dependence upon a source of growth factor(s). In response to Epo a population of the cells synthesize hemoglobin. Studies of globin expression indicated that the globin programs of B6SUTA cells are similar to those of erythroid progenitors at the period of transition 15 from the yolk sac to fetal liver erythropoiesis. TF-1 (6) it is a cell line of immature erythroid origin established from a patient with erythroleukemia. The cell line shows complete dependency on GM-CSF or IL-3. Epo sustains short-term growth of TF-1 and will induce hemoglobin synthesis in a very small population of cells (8%). Hemin 20 and  $\alpha$ -aminolevulinic acid induce hemoglobin synthesis in most of the cells.

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Human IL-3 will not bind the murine IL-3 receptor, therefore experiments that were done with B6SUTA cells measured only the functionality of the Epo moiety of the hybrid. B6SUTA cells are carried in murine IL-3. In each experiment, they are washed thoroughly and set up with growth factors at  $10^5$  cells/ml. Cell growth and hemoglobin content were monitored on days 3 and 6 of each experiment. Cells grown in the presence of concentrated (10X) CHO 30 conditioned medium (CM) containing IL-3:Epo Short at a final concentration equivalent to 4.8 units/ml of Epo grew as well as cells grown in an equivalent amount of recombinant human (rHu) Epo. The percentage of cells which synthesized hemoglobin in response to the CHO-IL-3:Epo Short CM was always four times that of cells exposed to rHu Epo. B6SUTA cells grown in the presence of rHu IL-3 and rHu Epo 35 grew as well as cells grown in the presence of IL-3:Epo Short and

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induced hemoglobin synthesis in the same percentage of cells as did rHu Epo. Cells exposed to recombinant murine IL-3 (rMu IL-3) and rHu Epo grew similarly to cells exposed to rMu IL-3 alone and neither effectively induced cells to synthesize hemoglobin. Concentrated control CHO CM did not support the growth of B6SUTA cells nor did it induce hemoglobin synthesis. CHO CM plus rHu Epo supported cell growth and hemoglobinization as well as CHO-IL-3:Epo Short CM.

CHO-IL-3:Epo Short CM as well as CHO-rHu IL-3 CM both supported growth of human TF-1 cells. Control CHO CM supported only limited growth of the TF-1 cells.

#### Discussion

The above-mentioned results demonstrate that a hybrid protein comprising two growth factors can be expressed in mammalian cell culture systems. In vitro assays of IL-3:Epo Short indicate that this hybrid protein has the activities of both IL-3 and Epo. The therapeutic application of such hybrid factors has advantages over using two factors separately simply in terms of patient administration, and moreover since the production, purification and formulation of one factor is less labor intensive than for two separate factors.

#### **Example 7**

Factor Dependent Cell Lines and Culture Media - The GM-CSF/IL-3/Epo dependent human TF-1 cell line and the G-CSF dependent murine NFS-60 cell line were grown and maintained as described (7,8.). The GM-CSF dependent human cell line AML 193 (9) was adapted for growth in IL-3 by continuous culture of the cells in RPMI-1640 plus 10% FCS supplemented with rHu IL-3 for 6 weeks. The TF-1 derived cell line, TF-136 was selected by continuous culture of the TF-1 line in RPMI-1640 plus 10% FCS supplemented with 5ng/ml of rHu IL-3 for 6 months, followed by single cell suspension cloning of the resultant IL-3 dependent cells. The Epo dependent murine cell line, FDC-P1/ER,

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was derived from the IL-3 dependent line, FDC-P1, by introduction of the murine Epo receptor into these cells. (10) FDC-P1/ER cells are maintained in RPMI-1640 plus 10% FCS supplemented with 1 unit/ml of rHu Epo. Recombinant human Epo was obtained from Ortho Biologicals, Inc (Raritan, NJ). Recombinant human IL-3, rHu G-CSF and rHu GM-CSF were purchased from R & D Systems (Minneapolis, MN).

5 Capture ELISA Assay - ELISA plate was coated with 5  $\mu$ g/200  $\mu$ l/well of goat anti-human IL-3 (R & D Systems) in PBS at 40°C overnight. Excess antibody was removed by washing with PBS. Blocking was carried out with 300  $\mu$ l/well of 1% non-fat milk in PBS for 1 hour at 37°C followed by washing with 0.05% Tween™ in PBS. Samples were then incubated with the IL-3 antibody for 1 hour at 37°C in 0.5% non-fat milk, 0.025% Tween™. Following extensive washing, the second antibody, a mouse 10 anti-Epo monoclonal (Genzyme, Cambridge, MA ), was added to the plate which was incubated for 1 hour at 37°C. The plate was washed and 15 incubated with conjugate antibody (Goat anti-mouse-horseradish peroxidase) for 30 minutes at 37°C. Color development was carried out with the addition of o-phenylenediamine/ $H_2O_2$  at room temperature (RT) 20 for 30 minutes. The reaction was stopped with 1N  $H_2SO_4$  and the samples were read at 495 nm.

25 Gene Amplification- CHO cell lines producing significant amounts of the hybrid growth factors were isolated and  $10^6$  cells were plated in a 75 mm T-flask in GMEM-S medium containing various concentrations of methionine sulphoximine (MSX), ranging between 100 $\mu$ M and 500 $\mu$ M. Colonies resistant to the highest MSX concentration (IL-3:Epo Flex 200 $\mu$ M; IL-3:Epo Short 250 $\mu$ M; Epo:IL-3 Short 250 $\mu$ M; IL-3:G-CSF 250 $\mu$ M) 30 were isolated and expanded. Those clones producing the highest levels of hybrid growth factors as determined by Epo or G-CSF ELISA assay (Amgen) were used for subsequent studies. IL-3:Epo Flex (clone 23-10); IL-3:Epo Short (clone 5-4); Epo:IL-3 Short (17-3-1).

35 Cell Proliferation Assays - Factor dependent cells were grown to stationary phase, washed, and incubated for 16 hours in media plus 10% FCS deprived of growth factor. The cells were plated at a

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concentration of  $2 \times 10^5$  cells/ml in a 96 well microtiter plate (200  $\mu$ l/well) with and without growth factor. Recombinant human growth factors were diluted into CHO conditioned medium (CM) before addition to cells. Following incubation for 42 hours, ( $^3$ H) thymidine (1  $\mu$ Ci/

5 well; New England Nuclear, Boston, MA) was added and the cells were incubated for another 6 hours. The cells were then hypotonically lysed and harvested onto glass fiber filters. The filters were washed with distilled water, dried and counted in liquid scintillation fluid.

10 Bone Marrow Cultures - Informed consent was obtained prior to aspirating bone marrow from normal volunteers. Aspirated bone marrow was diluted 1:1 in  $\alpha$ - medium without nucleosides containing preservative-free sodium heparin. A single cell suspension was prepared, layered, over an equal volume of Ficoll-Hypaque (sp gr 1.077 g/ml) and then centrifuged for 25 minutes at 1,500 rpm at 40°C. The light-density mononuclear cells were collected and washed and diluted to  $5 \times 10^5$  cells/ml with Iscove's modified Dulbecco's medium plus 20% modified FCS (Gibco BRL). Cells ( $1.25 \times 10^5$  /ml) were plated in 0.8% methylcellulose supplemented with various concentrations of rHu Epo, rHu IL-3 and hybrid growth factors. Cultures were incubated for either 7 or 14 days in a humidified atmosphere with 5%  $\text{CO}_2$  at 37°C. Colonies were counted at day 7 for CFU-E and at day 14 for BFU-E under an inverted microscope.

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25 Western Blot Analysis - CHO CM containing approximately 75 ng of IL-3:Epo fusion protein was electrophoresed on a 10-20% gradient SDS PAGE gel (Integrated Separations Systems) under reducing and denaturing conditions. Samples were loaded in 0.0625 M Tris-HCl (pH 6.8), 2% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.002% bromophenol blue following heat treatment at 100°C for 3 minutes. The proteins were transferred to nitrocellulose (Bio Rad) in 25 mM Tris, 129 mM glycine, pH 8.3, 20% methanol, at 150 V, constant power, for 90 minutes. The transfer efficiency was monitored by visual examination of the completeness of transfer of prestained molecular weight markers (Bio Rad). The nitrocellulose membrane was incubated in PBS containing 3% BSA for 1 hour at room temperature and subsequently washed in PBS

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containing 0.5% Tween (PBS-T) for 5 minutes at room temperature. The membrane was probed with primary anti-Epo anti-sera in 3% BSA in PBS. Excess antibody was removed by 3, 5 minute room temperature washes in PBS-T. The nitrocellulose membrane was then probed with a secondary antibody conjugate (Goat anti-Rabbit IgG/ Alkaline Phosphatase, Bio Rad) for 1 hour at room temperature. Excess secondary antibody was removed by two washes with PBS-T as above. Color development was carried out by incubation with color reagents (Bio Rad) in alkaline phosphatase buffer (100 mM Tris HCl, pH 9.5, 100 mM NaCl, 5 mM MgCl<sub>2</sub>). The reaction was stopped by immersion of the membrane in cold (4°C) distilled H<sub>2</sub>O.

#### Results and Discussion

Hybrid growth factor plasmid amplification. Individual transfected CHO cell clones producing significant amounts of the desired hybrid growth factor were identified by ELISA capture assay, Table II. The clones were plated out and placed in medium with increasing concentrations of MSX, ranging between 100  $\mu$ M and 500  $\mu$ M. Colonies surviving at the highest concentration of MSX were isolated and grown to confluence. Serum and drug-free medium was then added to the cells and collected after 4 days. At the time of collection fresh serum and drug-free medium was added to the cells. A total of 3 collections were taken. The amount of hybrid growth factor produced in the collections was determined by Epo or G-CSF ELISA assay (Table III) and appropriate collections were pooled. The pooled CM was used as a source of hybrid growth factors in all cellular assays.

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TABLE II

	<u>Hybrid Growth Factor</u>	<u>OD (495 nm)</u>
	CHO CM*	0.24
	IL-3:Epo Flex	2.44
5	IL-3:Epo Short	2.46
	Epo:IL-3 Short	1.59
	IL-3:G-CSF	2.40

\* Conditioned medium from CHO cells transfected with the vector  
 10 pE6.

TABLE III

	<u>Hybrid Growth Factor</u>	<u>Collection</u>	<u>Concentration (µg/ml)*</u>
15	IL-3:Epo Flex	1st	3.0
		2nd	4.2
		3rd	3.3
20	IL-3:Epo Short	1st	1.5
		2nd	5.8
		3rd	6.7
25	Epo:IL-3 Short	1st	26.7
		2nd	53.3
		3rd	58.7
25	IL-3:G-CSF	1st	2.2
		2nd	2.0
		3rd	2.0

\* Concentrations were determined by Epo and G-CSF ELISA Assay.

Detection of hybrid growth factor production. In order to confirm  
 30 that the IL-3 and Epo detected in the ELISA capture assays were being  
 produced in the form of a fusion protein, Western blot analysis was  
 performed. Conditioned medium from CHO cells transfected with IL-  
 3:Epo Flex cDNA (Figure 1, lane 2), IL-3:Epo Short cDNA (Figure 1,  
 lane 3) and Epo:IL-3 Short cDNA (Figure 1, lane 4) were probed with  
 35 mouse anti-Epo polyclonal anti-sera. Immunoreactive material  
 corresponding to a molecular weight of approximately 50,000 daltons.

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the expected size of the IL-3:Epo and Epo:IL-3 hybrid growth factors, was detected in each sample. Comparison with increasing concentrations of rHu Epo (Figure 1, lanes 6-10) indicated that the antibody used in this analysis recognizes the Epo moiety of the fusion proteins efficiently.

IL-3 bioactivity of the IL-3:Epo and Epo:IL-3 hybrid growth factors. To determine whether the IL-3 moiety of the IL-3:Epo and Epo:IL-3 hybrid growth factors was functional, its ability to support growth of the IL-3-dependent human cell line, AML193, was evaluated (Figure 2). As Epo does not support growth of these cells (Figure 2), only IL-3 activity was measured in this assay system. CHO CM containing rHu IL-3 and levels of hybrid growth factors sufficient to support maximal proliferation were added to the culture medium. The cells were then pulsed with (<sup>3</sup>H) thymidine and the radioactivity incorporated into the DNA was used as a measure of cell growth. Cells exposed to CHO CM containing no growth factors, supported the proliferation of AML193 cells to the same extent as did cells grown in medium alone. Each of the fusion proteins when present in excess, supported the growth of AML193 cells in a manner equivalent to that of rHu IL-3.

The functional activity of the IL-3 portion of the IL-3:Epo and Epo:IL-3 hybrid growth factors was further evaluated by comparing the fusion proteins to rHu IL-3 in dose response experiments (Figure 3). The incorporation of (<sup>3</sup>H) thymidine into AML193 DNA was again used as a measure of cell proliferation. When IL-3 was located at the N-terminus of the hybrid growth factor protein (IL-3:Epo), its ability to support AML193 proliferation was equivalent to that of rHu IL-3 (ED50 = 5 fmol/ml). Size (2 aa versus 23 aa) and flexibility of the linker did not greatly impact the function of the IL-3 moiety. However, when IL-3 was located at the C-terminus of the fusion protein (Epo:IL-3), its ability to support the growth of AML193 cells was less (ED50 = 200 fmol/ml) than that of rHu IL-3 and the IL-3:Epo hybrid factors. These results suggest that linkage of IL-3 at the N-terminus interferes with function while linkage at the C-terminus does not. It has previously been reported that modification of the C-terminus of

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murine IL-3 did not interfere with its activity (11). Therefore, it should be possible to target any molecule or compound of interest to cells expressing the IL-3 receptor through linkage to IL-3 at its C-terminus.

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Epo bioactivity of the IL-3:Epo and Epo:IL-3 hybrid growth factors. To determine whether the Epo moiety of the hybrid growth factors was functional, its ability to support the growth of the Epo-dependent murine cell line FDC-P1/ER, was evaluated (Figure 4). This line derived from FDC-P1 cells expresses the murine Epo receptor (10), and is dependent on either murine IL-3 or Epo (murine and human) for growth (Figure 4). As IL-3 is a species specific growth factor, murine IL-3-dependent cells do not respond to human IL-3 (12). Therefore, when using the FDC-P1/ER cell line to evaluate functionality, only the activity of the Epo moiety is measured. CHO CM containing rHu Epo and levels of hybrid growth factors sufficient to support maximal proliferation were added to the culture medium. The cells were then pulsed with (<sup>3</sup>H) thymidine and the radioactivity incorporated into the DNA was used as a measure of cell growth. Cells exposed to CHO CM which did not contain cytokines did not support the proliferation of FDC-P1/ER cells. Each of the fusion proteins when present in excess, stimulated the growth of FDC-P1/ER cells to the same extent as did rHu Epo. (Figure 4)

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The biological function of the Epo portion of the IL-3:Epo and Epo:IL-3 hybrid growth factors was further analyzed by comparing the fusion proteins to rHu Epo in dose response experiments (Figure 5). The incorporation of (<sup>3</sup>H) thymidine into FDC-P1/ER cells was used as a measure of cell proliferation. Each of the hybrid growth factors was equivalent to rHu Epo in ability to stimulate proliferation of FDC-P1/ER cells (ED<sub>50</sub> = 50 fmol/ml). Size (2-3 aa versus 23 aa) and flexibility of the linker, as well as the orientation of Epo within the protein (N-terminus versus C-terminus) did not alter function. Evidence exists suggesting that the N-terminus of Epo is not involved in receptor binding as a monoclonal antibody directed toward the N-terminus of Epo does not neutralize its activity (13). The results

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presented here suggest that linkage of Epo to a second protein does not impair its ability to bind its receptor or transduce a signal. Epo could therefore be useful as a carrier protein which would target a molecule or compound of interest to those cells expressing the Epo receptor.

IL-3 plus Epo bioactivity of the IL-3:Epo and Epo:IL-3 hybrid growth factors. In order to study the effects of IL-3 and Epo in combination, proliferation of a human cell line, TF-1 (7), dependent on IL-3 and Epo for growth was measured. This experiment was done on a cytokine weight basis and the results are represented on a molar basis (Figure 6). rHu IL-3 (R & D Systems) made in E. coli is nonglycosylated. rHu Epo and hybrid growth factors made in CHO cells are glycosylated. Therefore, when equal weights of the growth factors were added to the cell culture medium, approximately twice the number of unglycosylated molecules of IL-3 were added as compared to glycosylated Epo and hybrid growth factor molecules.

CHO CM containing rhu IL-3 plus rHu Epo and levels of hybrid growth factors which support suboptimal proliferation of TF-1 cells adapted for growth in IL-3 were added to the culture medium. Cell growth was monitored by radioactivity incorporated into the DNA. (Figure 6) The activities of IL-3 plus Epo were not synergistic in this cell line, nor were they additive. At these low levels, the activities of the IL-3:Epo Flex and IL-3:Epo Short fusion proteins were comparable to those of a mixture of the two cytokines. Epo:IL-3 Short activity was again reduced in comparison to that of the IL-3:Epo hybrid growth factors and the combination of IL-3 plus Epo. This is likely to be due to decreased IL-3 activity.

The biological activity of the IL-3:Epo and Epo:IL-3 hybrid growth factors was further evaluated in dose response experiments (Figures 7 & 8). TF-1 cells adapted for optimal growth in IL-3 were exposed to CHO CM containing hybrid growth factors (Figure 7). Each of the fusion proteins when present in excess were able to support growth of the cells to the same extent (Figure 7A). At lower doses, the IL-

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3:Epo Flex protein appeared to be slightly more potent than the short linkeder hybrids (IL-3:Epo Flex ED50 = 0.37 fmol/ml; IL-3:Epo Short ED50 = 0.75 fmol/ml; Epo:IL-3 Short ED50 = 0.9 fmol/ml) (Figure 7B). This result was more pronounced in experiments done with TF-1 cells adapted for optimal growth in GM-CSF (Figure 8). The IL-3:Epo Flex protein was dramatically more potent than the hybrid growth factors containing short linkers (IL-3:Epo Flex ED50 = 0.07 fmol/ml; IL-3:Epo Short and Epo:IL-3 Short ED50 = 0.75 fmol/ml (Figure 8B). When present in excess with the GM-CSF adapted TF-1 cells, each of the fusion proteins stimulated cell proliferation to a similar extent (Figure 8A). These results suggest that when IL-3 and Epo are fused, the 23 aa flexible linker allows more efficient receptor interaction than does a short (2-3 aa) linker.

It appears that induction of receptor expression is possible by growing a cell in the presence of a cytokine whose receptor it has the potential to express. An up regulation of Epo receptor expression has been reported in IL-3-dependent cells transferred into medium supplemented with Epo (14). Thus, it is likely that growing TF-1 cells in IL-3 or GM-CSF, preferentially increases the appearance of cell surface IL-3 or GM-CSF receptors. Several research groups (15-20) have observed a subset population of GM-CSF and IL-3 receptors on primary human cells and hematopoietic cell lines capable of binding both GM-CSF and IL-3. It has been suggested that a single accessory molecule preferentially interacts with this subset of GM-CSF/IL-3 receptors allowing the transduction of signal. Our results raise the possibility that GM-CSF could be inducing the expression of an accessory molecule in TF-1 cells which may be important for binding and could possibly link IL-3:Epo signal transduction. This protein could be identical to the GM-CSF/IL-3 receptor accessory protein.

Erythroid colony formation stimulated by IL-3:Epo and Epo:IL-3 hybrid growth factors. To assess the biological activity of the fusion proteins on normal hematopoietic progenitor cells, analysis of the formation of erythroid (BFU-E and CFU-E) colonies from nonadherent mononuclear human bone marrow cells was performed. (Table IV) As was

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observed with the cell lines, the Epo moiety of each of the fusion proteins was equally active (CFU-E formation) on bone marrow progenitor cells. The IL-3:Epo Flex protein was the most active hybrid growth factor while the Epo:IL-3 Short protein was the least. (It stimulated two-thirds the number of BFU-E as did the IL-3:Epo Flex.) These results suggest that the IL-3:Epo Flex fusion protein may have significant clinical benefits where indications for combination therapy with IL-3 and Epo may prove efficacious.

10

TABLE IV

	<u>Growth Factors</u>	<u>BFU-E<sup>a,b</sup></u>	<u>CFU-E<sup>c</sup></u>
	No Factors	-	-
	0.2 pmol/ml IL-3	-	-
15	0.1 pmol/ml Epo	+	+++
	0.2 pmol/ml IL-3 &		
	0.1 pmol/ml Epo	+++	+++
	0.1 pmol/ml IL-3:Epo Flex	+++	+++
	0.1 pmol/ml IL-3:Epo Short	++	+++
20	0.1 pmol/ml Epo:IL-3 Short	+	+++

a Mononuclear human bone marrow cells were used as a target cell population.

b BFU-E were counted 14 days after plating.

25 c CFU-E were counted 7 days after plating.

IL-3 bioactivity of the IL-3:G-CSF hybrid growth factor. To determine whether the IL-3 moiety of the IL-3:G-CSF hybrid growth factor was functional, its ability to support growth of the IL-3-dependent human cell line TF-1, was evaluated in a dose response experiment (Figure 9). Quantitation of IL-3:G-CSF protein in CHO CM was performed using a G-CSF ELISA assay in which the standard is unglycosylated G-CSF. Since the IL-3:G-CSF fusion protein is glycosylated, measurements are approximate. G-CSF does not support growth of TF-1 cells (Figure 9), therefore, the only activity measured in this assay system was IL-3.

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5 CHO CM containing rhu IL-3, rHu G-CSF, and IL-3:G-CSF hybrid growth factor were added to the culture medium. The radioactivity incorporated into the DNA was used as a measure of cell proliferation. CHO CM did not support growth of TF-1 cells. The mixture of rhu IL-3 plus rHu G-CSF stimulated proliferation to the same extent as did rhu IL-3 alone. The IL-3:G-CSF hybrid growth factor induced a dose response similar to that observed with IL-3.

10 G-CSF bioactivity of the IL-3:G-CSF hybrid growth factor. To evaluate the biological function of the G-CSF moiety of the IL-3:G-CSF hybrid growth factor, its ability to stimulate proliferation of the murine cell line, NSF-60, was tested. (Figure 10) G-CSF, unlike IL-3 is not species specific, therefore, human G-CSF will actively support growth of murine cells (21). Cells exposed to CHO CM containing no growth factors, supported the proliferation of NSF-60 cells to the same extent as did cells grown in medium alone. The IL-3:G-CSF hybrid growth factor stimulated growth in a dose dependent manner equivalent to that observed with G-CSF.

20 IL-3 plus G-CSF bioactivity of the IL-3:G-CSF hybrid growth factor. The biological function of the IL-3:G-CSF hybrid growth factor was evaluated by its ability to support growth of an IL-3-, G-CSF-dependent human cell line, AML193. CHO CM containing rHu IL-3, rHu G-CSF and IL-3:G-CSF hybrid growth factor were added to the culture medium. Cell proliferation was monitored by incorporation of radioactivity into the DNA. (Figure 11). Both IL-3 and G-CSF supported growth of this cell line in a dose dependent manner. The two cytokine activities were not synergistic, nor were they additive. The IL-3:G-CSF hybrid growth factor stimulated AML193 proliferation to a greater extent than did the mixture of the two cytokines.

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REFERENCES

1. Sonoda, et al., Proc. Natl. Acad. Sci. USA, 85:4630-4364 (1988).
- 5 2. Migliaccio, et al., Blood, Vol. 72, No. 3, 844-851 (1988).
3. Sieff, et al., Blood, Vol.73, No. 3, 688-693 (1989).
4. Fraser, et al., Exp. Hematol., 16: 769-775 (1988).
- 10 5. Enver, et al, Proc.Natl. Acad. Sci. USA, 85:9091-9095 (1988).
6. Kitamura, et al., J. Physiol., 140:323-334 (1989).
- 15 7. Kitamura, et al., J. Cell. Physiol., 140 : 323-334 (1989).
8. Holmes, et al., Proc. Natl. Acad. Sci. USA, 82 : 6687-6691 (1985).
- 20 9. Lange, et al., Blood, 70 : 192-199 (1987).
10. Carroll, et al., J. Biol. Chem., in press (1991).
11. Dunbar, et al., Science, 245 : 1493-1496 (1989).
- 25 12. Yang, et al., Cell, 47 : 3-10 (1986).
13. Sue, et al., Proc. Natl. Acad. Sci. USA, 80 : 3651-3655 (1983).
- 30 14. Sakaguchi, et al., Biochem. Biophys. Res. Commun., 146 : 7-12 (1987).
15. Park, et al., J. Biol. Chem., 264 : 5420-5427 (1989).
- 35 16. Gesner, et al., J. Cell. Physiol., 136 : 493-499 (1988).

- 29 -

17. Elliot, et al., Blood, 74 : 2349-2359 (1989).
18. Lopez, et al., Proc. Natl. Acad. Sci. USA, 86 : 7022-7026 (1989).
- 5 19. Budel, et al., Blood, 75 : 1439-1445 (1990).
20. Cannistra, et al., J. Biol. Chem., 265 : 12656-12663 (1990).
- 10 21. Souza, et al., Science, 232 : 61-65 (1986).
22. Umemura et al., Proc. Natl. Acad. Sci. USA, 85 : 9278-9282 (1988).
- 15 23. Udupa, et al., Blood, 53 : 1164-1171 (1979).
24. Papayannopoulou, et al., J. Clin. Invest., 51 : 1179-1185 (1972).
- 20 25. Migliaccio, et al., Blood, 72 : 944-951 (1988).

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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- (ii) TITLE OF INVENTION: HYBRID GROWTH FACTORS
- (iii) NUMBER OF SEQUENCES: 44
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- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
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  - (C) TELEX: 844-481

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 133 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys  
1 5 10 15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu  
20 25 30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu  
35 40 45

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
50 55 60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn  
65 70 75 80

Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro  
85 90 95

Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr  
100 105 110

Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu  
115 120 125

Ser Leu Ala Ile Phe  
130

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

- 32 -

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys  
1 5 10 15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu  
20 25 30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu  
35 40 45

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
50 55 60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys  
65 70 75

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 166 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu  
1 5 10 15

Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His  
20 25 30

Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe  
35 40 45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp  
50 55 60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu  
65 70 75 80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp  
85 90 95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu  
100 105 110

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Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala  
115 120 125  
Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val  
130 135 140  
Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala  
145 150 155 160  
Cys Arg Thr Gly Asp Arg  
165

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 155 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala  
1 5 10 15  
Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu Asn  
20 25 30  
Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg Met  
35 40 45  
Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu Leu  
50 55 60  
Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser Gln  
65 70 75 80  
Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly Leu  
85 90 95  
Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu Ala  
100 105 110

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Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile Thr  
115 120 125  
Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu Arg  
130 135 140  
Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys  
145 150 155

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 174 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys  
1 5 10 15  
Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln  
20 25 30  
Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val  
35 40 45  
Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys  
50 55 60  
Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser  
65 70 75 80  
Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser  
85 90 95  
Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp  
100 105 110  
Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro  
115 120 125  
Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe  
130 135 140

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Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe  
145 150 155 160  
Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro  
165 170

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 302 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys  
1 5 10 15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu  
20 25 30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu  
35 40 45

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
50 55 60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn  
65 70 75 80

Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro  
85 90 95

Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr  
100 105 110

Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Thr Thr Leu  
115 120 125

Ser Leu Ala Ile Phe Leu Asp Met Ala Pro Pro Arg Leu Ile Cys Asp  
130 135 140

Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn  
145 150 155 160

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Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu Asn Ile Thr  
 165 170 175

Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg Met Glu Val  
 180 185 190

Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu Leu Ser Glu  
 195 200 205

Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser Gln Pro Trp  
 210 215 220

Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly Leu Arg Ser  
 225 230 235 240

Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu Ala Ile Ser  
 245 250 255

Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile Thr Ala Asp  
 260 265 270

Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu Arg Gly Lys  
 275 280 285

Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp Arg  
 290 295 300

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 321 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys  
 1 5 10 15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu  
 20 25 30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu  
 35 40 45

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Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
50 55 60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn  
65 70 75 80

Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro  
85 90 95

Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr  
100 105 110

Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu  
115 120 125

Ser Leu Ala Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly  
130 135 140

Gly Gly Ser Ala Leu Ala Ile Phe Leu Asp Met Ala Pro Pro Arg Leu  
145 150 155 160

Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu  
165 170 175

Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu  
180 185 190

Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg  
195 200 205

Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu  
210 215 220

Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser  
225 230 235 240

Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly  
245 250 255

Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu  
260 265 270

Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile  
275 280 285

Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu  
290 295 300

Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp  
305 310 315 320

Arg

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 303 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu  
1 5 10 15

Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His  
20 25 30

Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe  
35 40 45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp  
50 55 60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu  
65 70 75 80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp  
85 90 95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu  
100 105 110

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala  
115 120 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val  
130 135 140

Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala  
145 150 155 160

Cys Arg Thr Gly Asp Arg Ala Ala Met Ala Pro Met Thr Gln Thr  
165 170 175

Thr Ser Leu Lys Thr Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu  
180 185 190

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Ile Ile Thr His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn  
195 200 205  
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg  
210 215 220  
Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn  
225 230 235 240  
Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro  
245 250 255  
Leu Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly  
260 265 270  
Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu  
275 280 285  
Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe  
290 295 300

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 322 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu  
1 5 10 15  
Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His  
20 25 30  
Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe  
35 40 45  
Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp  
50 55 60  
Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu  
65 70 75 80

- 40 -

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp  
85 90 95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu  
100 105 110

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala  
115 120 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val  
130 135 140

Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala  
145 150 155 160

Cys Arg Thr Gly Asp Arg Ala Ala Ala Ser Gly Gly Gly Gly Ser Gly  
165 170 175

Gly Gly Gly Ser Gly Gly Gly Ser Ala Ala Ala Met Ala Pro Met  
180 185 190

Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys Ser Asn Met  
195 200 205

Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu Pro Leu Leu  
210 215 220

Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn  
225 230 235 240

Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala Val Lys Ser  
245 250 255

Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro  
260 265 270

Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile  
275 280 285

Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu  
290 295 300

Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala  
305 310 315 320

Ile Phe

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 317 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn Cys  
1 5 10 15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu  
20 25 30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu  
35 40 45

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala  
50 55 60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn  
65 70 75 80

Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro  
85 90 95

Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr  
100 105 110

Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu  
115 120 125

Ser Leu Ala Ile Phe Leu Glu Ala Ala Ala Ser Leu Pro Ala Met Thr  
130 135 140

Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys  
145 150 155 160

Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu  
165 170 175

Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu  
180 185 190

Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro  
195 200 205

Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly  
210 215 220

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Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro  
225 230 235 240

Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe  
245 250 255

Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala  
260 265 270

Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln  
275 280 285

Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu  
290 295 300

Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro  
305 310 315

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 994 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 14..977

(ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 71..977

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AATTGCCGCC ACC ATG AGC CGC CTG CCC GTC CTG CTC CTG CTC CAA CTC 49  
Met Ser Arg Leu Pro Val Leu Leu Leu Gln Leu  
-19 -15 -10

CTG GTC CGC CCC GGA CTC CAA GCT CCC ATG ACC CAG ACA ACT AGT TTG 97  
Leu Val Arg Pro Gly Leu Gln Ala Pro Met Thr Gln Thr Thr Ser Leu  
-5 1 5

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AAG ACA AGC TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA	145
Lys Thr Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr	
10 15 20 25	
CAC TTA AAC GAG CCA CCT TTG CCT TTG CTG GAC TTC AAC AAC CTC AAT	193
His Leu Asn Glu Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn	
30 35 40	
GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT AAC CTT CGA AGG CCA AAC	241
Gly Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn	
45 50 55	
CTG GAG GCA TTC AAC AGG GCT GTC AAG AGT TTA CAG AAT GCA TCA GCA	289
Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala	
60 65 70	
ATT GAG AGC ATT CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG	337
Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr	
75 80 85	
GCC GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC TGG AAT	385
Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp Asn	
90 95 100 105	
GAA TTC CGG AGG AAA CTG ACG TTC TAT CTG AAA ACC CTT GAG AAT GCG	433
Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala	
110 115 120	
CAG GCT CAA CAG ACG ACT TTG TCG CTA GCG ATC TTT CTA GAC ATG GCC	481
Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe Leu Asp Met Ala	
125 130 135	
CCA CCA CGC CTC ATC TGT GAC AGC CGA GTC CTG GAG AGG TAC CTC TTG	529
Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu	
140 145 150	
GAG GCC AAG GAG GCC GAG AAT ATC ACG ACG GGC TGT GCT GAA CAC TGC	577
Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys	
155 160 165	
AGC TTG AAT GAG AAT ATC ACT GTC CCA GAC ACC AAA GTT AAT TTC TAC	625
Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr	
170 175 180 185	
GCG TGG AAG AGG ATG GAG GTC GGC CAG CAG GCC GTA GAA GTC TGG CAG	673
Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln	
190 195 200	
GGC CTG GCC CTG CTG TCG GAA GCT GTC CTG CGG GGC CAG GCC CTG TTG	721
Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu	
205 210 215	

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GTC AAC TCG AGC CAG CCG TGG GAG CCC CTG CAA CTG CAT GTG GAT AAA	769
Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys	
220 225 230	
GCC GTC AGT GGC CTT CGC AGC CTC ACC ACT CTG CTT CGG GCT CTG GGA	817
Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly	
235 240 245	
GCT CAG AAG GAA GCC ATC TCC CCT CCA GAT GCG GCC TCA GCT GCT CCA	865
Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro	
250 255 260 265	
CTC CGA ACA ATC ACT GCT GAC ACT TTC CGC AAA CTC TTC CGA GTC TAC	913
Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr	
270 275 280	
TCC AAT TTC CTC CGG GGA AAG CTG AAG CTG TAC ACA GGG GAG GCA TGC	961
Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys	
285 290 295	
AGG ACA GGG GAC AGA T GATAAGGATC CGAATTC	994
Arg Thr Gly Asp Arg	
300	

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1015 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 8..998

## (ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 89..998

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TCGAGCC ATG GGG GTG CAC GAA TGT CCT GCC TGG CTG TGG CTT CTC CTG	49
Met Gly Val His Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu	
-27 -25 -20 -15	

- 45 -

TCC CTG CTG TCG CTC CCT CTG GGC CTC CCA GTC CTG GGC GCC CCA CCA	97
Ser Leu Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro	
-10	-5
	1
CGC CTC ATC TGT GAC AGC CGA GTC CTG GAG AGG TAC CTC TTG GAG GCC	145
Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala	
5	10
	15
AAG GAG GCC GAG AAT ATC ACG ACG GGC TGT GCT GAA CAC TGC AGC TTG	193
Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu	
20	25
	30
	35
AAT GAG AAT ATC ACT GTC CCA GAC ACC AAA GTT AAT TTC TAC GCG TGG	241
Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp	
40	45
	50
AAG AGG ATG GAG GTC GGC CAG CAG GCC GTA GAA GTC TGG CAG GGC CTG	289
Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu	
55	60
	65
GCC CTG CTG TCG GAA GCT GTC CTG CGG GGC CAG GCC CTG TTG GTC AAC	337
Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn	
70	75
	80
TCG AGC CAG CCG TGG GAG CCC CTG CAA CTG CAT GTG GAT AAA GCC GTC	385
Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val	
85	90
	95
AGT GGC CTT CGC AGC CTC ACC ACT CTG CTT CGG GCT CTG GGA GCT CAG	433
Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln	
100	105
	110
	115
AAG GAA GCC ATC TCC CCT CCA GAT GCG GCC TCA GCT GCT CCA CTC CGA	481
Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg	
120	125
	130
ACA ATC ACT GCT GAC ACT TTC CGC AAA CTC TTC CGA GTC TAC TCC AAT	529
Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn	
135	140
	145
TTC CTC CGG GGA AAG CTG AAG CTG TAC ACA GGG GAG GCA TGC AGG ACA	577
Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr	
150	155
	160
GGG GAC AGA GCG GCC GCC ATG GCT CCC ATG ACC CAG ACA ACT AGT TTG	625
Gly Asp Arg Ala Ala Ala Met Ala Pro Met Thr Gln Thr Thr Ser Leu	
165	170
	175
AAG ACA AGC TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA	673
Lys Thr Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr	
180	185
	190
	195

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CAC TTA AAC GAG CCA CCT TTG CCT TTG CTG GAC TTC AAC AAC CTC AAT	721
His Leu Asn Glu Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn	
200 205 210	
GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT AAC CTT CGA AGG CCA AAC	769
Gly Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn	
215 220 225	
CTG GAG GCA TTC AAC AGG GCT GTC AAG AGT TTA CAG AAT GCA TCA GCA	817
Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala	
230 235 240	
ATT GAG AGC ATT CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG	865
Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr	
245 250 255	
GCC GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC TGG AAT	913
Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp Asn	
260 265 270 275	
GAA TTC CGG AGG AAA CTG ACG TTC TAT CTG AAA ACC CTT GAG AAT GCG	961
Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala	
280 285 290	
CAG GCT CAA CAG ACG ACT TTG TCG CTA GCG ATC TTT T AGTAAGGATC	1008
Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe	
295 300	
CGAATTC	1015

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1039 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 14..1021

(ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 71..1021

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AATTGCCGCC	ACC	ATG	AGC	CGC	CTG	CCC	GTC	CTG	CTC	CTG	CTC	CAA	CTC	49		
Met	Ser	Arg	Leu	Pro	Val	Leu	Leu	Leu	Leu	Gln	Leu					
-19							-15					-10				
CTG	GTC	CGC	CCC	GGA	CTC	CAA	GCT	CCC	ATG	ACC	CAG	ACA	ACT	AGT	TTG	97
Leu	Val	Arg	Pro	Gly	Leu	Gln	Ala	Pro	Met	Thr	Gln	Thr	Thr	Ser	Leu	
-5							1					5				
AAG	ACA	AGC	TGG	GTT	AAC	TGC	TCT	AAC	ATG	ATC	GAT	GAA	ATT	ATA	ACA	145
Lys	Thr	Ser	Trp	Val	Asn	Cys	Ser	Asn	Met	Ile	Asp	Glu	Ile	Ile	Thr	
10							15				20		25			
CAC	TTA	AAC	GAG	CCA	CCT	TTG	CCT	TTG	CTG	GAC	TTC	AAC	AAC	CTC	AAT	193
His	Leu	Asn	Glu	Pro	Pro	Leu	Pro	Leu	Leu	Asp	Phe	Asn	Asn	Leu	Asn	
							30				35		40			
GGG	GAA	GAC	CAA	GAC	ATT	CTG	ATG	GAA	AAT	AAC	CTT	CGA	AGG	CCA	AAC	241
Gly	Glu	Asp	Gln	Asp	Ile	Leu	Met	Glu	Asn	Asn	Leu	Arg	Arg	Pro	Asn	
45							50					55				
CTG	GAG	GCA	TTC	AAC	AGG	GCT	GTC	AAG	AGT	TTA	CAG	AAT	GCA	TCA	GCA	289
Leu	Glu	Ala	Phe	Asn	Arg	Ala	Val	Lys	Ser	Leu	Gln	Asn	Ala	Ser	Ala	
60							65					70				
ATT	GAG	AGC	ATT	CTT	AAA	AAT	CTC	CTG	CCA	TGT	CTG	CCC	CTG	GCC	ACG	337
Ile	Glu	Ser	Ile	Leu	Lys	Asn	Leu	Leu	Pro	Cys	Leu	Pro	Leu	Ala	Thr	
75							80					85				
GCC	GCA	CCC	ACG	CGA	CAT	CCA	ATC	CAT	ATC	AAG	GAC	GGT	GAC	TGG	AAT	385
Ala	Ala	Pro	Thr	Arg	His	Pro	Ile	His	Ile	Lys	Asp	Gly	Asp	Trp	Asn	
90							95				100		105			
GAA	TTC	CGG	AGG	AAA	CTG	ACG	TTC	TAT	CTG	AAA	ACC	CTT	GAG	AAT	GCG	433
Glu	Phe	Arg	Arg	Lys	Leu	Thr	Phe	Tyr	Leu	Lys	Thr	Leu	Glu	Asn	Ala	
110							115						120			
CAG	GCT	CAA	CAG	ACG	ACT	TTG	TCG	CTA	GCG	ATC	TTT	CTA	GAA	GCG	GCC	481
Gln	Ala	Gln	Gln	Thr	Thr	Leu	Ser	Leu	Ala	Ile	Phe	Leu	Glu	Ala	Ala	
125							130						135			
GCA	AGC	TTA	CCT	GCC	ATG	ACC	CCC	CTG	GGC	CCT	GCC	AGC	TCC	CTG	CCC	529
Ala	Ser	Leu	Pro	Ala	Met	Thr	Pro	Leu	Gly	Pro	Ala	Ser	Ser	Leu	Pro	
140							145					150				
CAG	AGC	TTC	CTG	CTC	AAG	TGC	TTA	GAG	CAA	GTG	AGG	AAG	ATC	CAG	GGC	577
Gln	Ser	Phe	Leu	Leu	Lys	Cys	Leu	Glu	Gln	Val	Arg	Lys	Ile	Gln	Gly	
155							160					165				
GAT	GGC	GCA	GCG	CTC	CAG	GAG	AAG	CTG	TGT	GCC	ACC	TAC	AAG	CTG	TGC	625
Asp	Gly	Ala	Ala	Leu	Gln	Glu	Lys	Leu	Cys	Ala	Thr	Tyr	Lys	Leu	Cys	
170							175				180		185			

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CAC CCC GAG GAG CTG GTG CTG CTC GGA CAC TCT CTG GGC ATC CCC TGG	673
His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp	
190 195 200	
GCT CCC CTG AGC TCC TGC CCC AGC CAG GCC CTG CAG CTG GCA GGC TGC	721
Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys	
205 210 215	
TTG AGC CAA CTC CAT AGC GGC CTT TTC CTC TAC CAG GGG CTC CTG CAG	769
Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln	
220 225 230	
GCC CTG GAA GGG ATA TCC CCC GAG TTG GGT CCC ACC TTG CAC ACA CTG	817
Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu His Thr Leu	
235 240 245	
CAG CTG GAC GTC GCC GAC TTT GCC ACC ACC ATC TGG CAG CAG ATG GAA	865
Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu	
250 255 260 265	
GAA CTG GGA ATG GCC CCT GCC CTG CAG CCC ACC CAG GGT GCC ATG CCG	913
Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro	
270 275 280	
GCC TTC GCC TCT GCT TTC CAG CGC CGG GCA GGA GGG GTC CTG GTT GCT	961
Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala	
285 290 295	
AGC CAT CTG CAG AGC TTC CTG GAG GTG TCG TAC CGC GTT CTA CGC CAC	1009
Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His	
300 305 310	
CTT GCG CAG CCC TGATAAGGAT CCGAATTG	1039
Leu Ala Gln Pro	
315	

## (2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1051 base pairs.
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

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(A) NAME/KEY: CDS  
 (B) LOCATION: 14..1033

## (ix) FEATURE:

(A) NAME/KEY: mat\_peptide  
 (B) LOCATION: 71..1033

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AATTGCCGCC	ACC	ATG	AGC	CGC	CTG	CCC	GTC	CTG	CTC	CTG	CTC	CAA	CTC	49		
Met	Ser	Arg	Leu	Pro	Val	Leu	Leu	Leu	Leu	Gln	Leu					
-19							-15			-10						
CTG	GTC	CGC	CCC	GGA	CTC	CAA	GCT	CCC	ATG	ACC	CAG	ACA	ACT	AGT	TTG	97
Leu	Val	Arg	Pro	Gly	Leu	Gln	Ala	Pro	Met	Thr	Gln	Thr	Thr	Ser	Leu	
-5							1			5						
AAG	ACA	AGC	TGG	GTT	AAC	TGC	TCT	AAC	ATG	ATC	GAT	GAA	ATT	ATA	ACA	145
Lys	Thr	Ser	Trp	Val	Asn	Cys	Ser	Asn	Met	Ile	Asp	Glu	Ile	Ile	Thr	
10							15			20			25			
CAC	TTA	AAC	GAG	CCA	CCT	TTG	CCT	TTG	CTG	GAC	TTC	AAC	AAC	CTC	AAT	193
His	Leu	Asn	Glu	Pro	Pro	Leu	Pro	Leu	Leu	Asp	Phe	Asn	Asn	Leu	Asn	
30							35			40						
GGG	GAA	GAC	CAA	GAC	ATT	CTG	ATG	GAA	AAT	AAC	CTT	CGA	AGG	CCA	AAC	241
Gly	Glu	Asp	Gln	Asp	Ile	Leu	Met	Glu	Asn	Asn	Leu	Arg	Arg	Pro	Asn	
45							50			55						
CTG	GAG	GCA	TTC	AAC	AGG	GCT	GTC	AAG	AGT	TTA	CAG	AAT	GCA	TCA	GCA	289
Leu	Glu	Ala	Phe	Asn	Arg	Ala	Val	Lys	Ser	Leu	Gln	Asn	Ala	Ser	Ala	
60							65			70						
ATT	GAG	AGC	ATT	CTT	AAA	AAT	CTC	CTG	CCA	TGT	CTG	CCC	CTG	GCC	ACG	337
Ile	Glu	Ser	Ile	Leu	Lys	Asn	Leu	Leu	Pro	Cys	Leu	Pro	Leu	Ala	Thr	
75							80			85						
GCC	GCA	CCC	ACG	CGA	CAT	CCA	ATC	CAT	ATC	AAG	GAC	GGT	GAC	TGG	AAT	385
Ala	Ala	Pro	Thr	Arg	His	Pro	Ile	His	Ile	Lys	Asp	Gly	Asp	Trp	Asn	
90							95			100			105			
GAA	TTC	CGG	AGG	AAA	CTG	ACG	TTC	TAT	CTG	AAA	ACC	CTT	GAG	AAT	GCG	433
Glu	Phe	Arg	Arg	Lys	Leu	Thr	Phe	Tyr	Leu	Lys	Thr	Leu	Glu	Asn	Ala	
110							115			120						
CAG	GCT	CAA	CAG	ACG	ACT	TTG	TCG	CTA	GCG	TCC	GGA	GGC	GGT	GGC	TCG	481
Gln	Ala	Gln	Gln	Thr	Thr	Leu	Ser	Leu	Ala	Ser	Gly	Gly	Gly	Gly	Ser	
125							130			135						
GGC	GGT	GGC	GGC	TCG	GGT	GGC	GGC	GGC	TCT	GCG	CTA	GCG	ATC	TTT	CTA	529
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Leu	Ala	Ile	Phe	Leu	
140							145			150						

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GAC ATG GCC CCA CCA CGC CTC ATC TGT GAC AGC CGA GTC CTG GAG AGG	577
Asp Met Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg	
155 160 165	
TAC CTC TTG GAG GCC AAG GAG GCC GAG AAT ATC ACG ACG GGC TGT GCT	625
Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala	
170 175 180 185	
GAA CAC TGC AGC TTG AAT GAG AAT ATC ACT GTC CCA GAC ACC AAA GTT	673
Glu His Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val	
190 195 200	
AAT TTC TAC GCG TGG AAG AGG ATG GAG GTC GGC CAG CAG GCC GTA GAA	721
Asn Phe Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu	
205 210 215	
GTC TGG CAG GGC CTG GCC CTG CTG TCG GAA GCT GTC CTG CGG GGC CAG	769
Val Trp Gln Gly Leu Ala Leu Ser Glu Ala Val Leu Arg Gly Gln	
220 225 230	
GCC CTG TTG GTC AAC TCG AGC CAG CCG TGG GAG CCC CTG CAA CTG CAT	817
Ala Leu Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His	
235 240 245	
GTG GAT AAA GCC GTC AGT GGC CTT CGC AGC CTC ACC ACT CTG CTT CGG	865
Val Asp Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg	
250 255 260 265	
GCT CTG GGA GCT CAG AAG GAA GCC ATC TCC CCT CCA GAT GCG GCC TCA	913
Ala Leu Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser	
270 275 280	
GCT GCT CCA CTC CGA ACA ATC ACT GCT GAC ACT TTC CGC AAA CTC TTC	961
Ala Ala Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe	
285 290 295	
CGA GTC TAC TCC AAT TTC CTC CGG GGA AAG CTG AAG CTG TAC ACA GGG	1009
Arg Val Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly	
300 305 310	
GAG GCA TGC AGG ACA GGG GAC AGA TGATAAGGAT CCGAATTTC	1051
Glu Ala Cys Arg Thr Gly Asp Arg	
315 320	

## (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1072 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 8..1054

(ix) FEATURE:

(A) NAME/KEY: mat\_peptide  
(B) LOCATION: 89..1054

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCGAGCC ATG GGG GTG CAC GAA TGT CCT GCC TGG CTG TGG CTT CTC CTG	49
Met Gly Val His Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu	
-27 -25 -20 -15	
TCC CTG CTG TCG CTC CCT CTG GGC CTC CCA GTC CTG GGC GCC CCA CCA	97
Ser Leu Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro	
-10 -5 1	
CGC CTC ATC TGT GAC AGC CGA GTC CTG GAG AGG TAC CTC TTG GAG GCC	145
Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala	
5 10 15	
AAG GAG GCC GAG AAT ATC ACG ACG GGC TGT GCT GAA CAC TGC AGC TTG	193
Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu	
20 25 30 35	
AAT GAG AAT ATC ACT GTC CCA GAC ACC AAA GTT AAT TTC TAC GCG TGG	241
Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp	
40 45 50	
AAG AGG ATG GAG GTC GGC CAG CAG GCC GTA GAA GTC TGG CAG GGC CTG	289
Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu	
55 60 65	
GCC CTG CTG TCG GAA GCT GTC CTG CGG GGC CAG GCC CTG TTG GTC AAC	337
Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn	
70 75 80	
TCG AGC CAG CCG TGG GAG CCC CTG CAA CTG CAT GTG GAT AAA GCC GTC	385
Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val	
85 90 95	
AGT GGC CTT CGC AGC CTC ACC ACT CTG CTT CGG GCT CTG GGA GCT CAG	433
Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln	
100 105 110 115	

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AAG GAA GCC ATC TCC CCT CCA GAT GCG GCC TCA GCT GCT CCA CTC CGA	481
Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg	
120 125 130	
ACA ATC ACT GCT GAC ACT TTC CGC AAA CTC TTC CGA GTC TAC TCC AAT	529
Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn	
135 140 145	
TTC CTC CGG GGA AAG CTG AAG CTG TAC ACA GGG GAG GCA TGC AGG ACA	577
Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr	
150 155 160	
GGG GAC AGA GCG GCC GCC TCC GGA GGC GGT GGC TCG GGC GGT GGC GGC	625
Gly Asp Arg Ala Ala Ser Gly Gly Gly Ser Gly Gly Gly	
165 170 175	
TCG GGT GGC GGC GGC TCT GCG GCC GCC ATG GCT CCC ATG ACC CAG ACA	673
Ser Gly Gly Gly Ser Ala Ala Ala Met Ala Pro Met Thr Gln Thr	
180 185 190 195	
ACT AGT TTG AAG ACA AGC TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA	721
Thr Ser Leu Lys Thr Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu	
200 205 210	
ATT ATA ACA CAC TTA AAC GAG CCA CCT TTG CCT TTG CTG GAC TTC AAC	769
Ile Ile Thr His Leu Asn Glu Pro Pro Leu Pro Leu Leu Asp Phe Asn	
215 220 225	
AAC CTC AAT GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT AAC CTT CGA	817
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg	
230 235 240	
AGG CCA AAC CTG GAG GCA TTC AAC AGG GCT GTC AAG AGT TTA CAG AAT	865
Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn	
245 250 255	
GCA TCA GCA ATT GAG AGC ATT CTT AAA AAT CTC CTG CCA TGT CTG CCC	913
Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro	
260 265 270 275	
CTG GCC ACG GCC GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT	961
Leu Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly	
280 285 290	
GAC TGG AAT GAA TTC CGG AGG AAA CTG ACG TTC TAT CTG AAA ACC CTT	1009
Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu	
295 300 305	
GAG AAT GCG CAG GCT CAA CAG ACG ACT TTG TCG CTA GCG ATC TTT	1054
Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe	
310 315 320	
TAGTAAGGAT CCGAATT	1072

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(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 429 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

( ix ) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 10..411

( ix ) FEATURE:

(A) NAME/KEY: mat\_peptide  
(B) LOCATION: 13..411

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AAGCTTACC ATG GCT CCC ATG ACC CAG ACA ACT AGT TTG AAG ACA AGC  
Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser  
-1 1 5 10

48

TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA CAC TTA AAC  
Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Asn  
15 20 25

96

GAG CCA CCT TTG CCT TTG CTG GAC TTC AAC AAC CTC AAT GGG GAA GAC  
 Glu Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp  
 30 35 40

144

CAA GAC ATT CTG ATG GAA AAT AAC CTT CGA AGG CCA AAC CTG GAG GCA  
Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala  
45 50 55 60

192

TTC AAC AGG GCT GTC AAG AGT TTA CAG AAT GCA TCA GCA ATT GAG AGC  
 Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser  
 65 70 75

240

ATT CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG GCC GCA CCC  
 Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro  
 80 85 90

288

ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC TGG AAT GAA TTC CGG  
 Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg  
 95 100 105

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AGG AAA CTG ACG TTC TAT CTG AAA ACC CTT GAG AAT GCG CAG GCT CAA	384
Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln	
110 115 120	
CAG ACG ACT TTG TCG CTA GCG ATC TTT TAGTAAGGAT CCGAATT	429
Gln Thr Thr Leu Ser Leu Ala Ile Phe	
125 130	

## (2) INFORMATION FOR SEQ ID NO:17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 532 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 14..514

## (ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 17..514

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AAGCTTACCT GCC ATG GCC CCA CCA CGC CTC ATC TGT GAC AGC CGA GTC	49
Met Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val	
-1 1 5 10	
CTG GAG AGG TAC CTC TTG GAG GCC AAG GAG GCC GAG AAT ATC ACG ACG	97
Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr	
15 20 25	
GGC TGT GCT GAA CAC TGC AGC TTG AAT GAG AAT ATC ACT GTC CCA GAC	145
Gly Cys Ala Glu His Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp	
30 35 40	
ACC AAA GTT AAT TTC TAC GCG TGG AAG AGG ATG GAG GTC GGC CAG CAG	193
Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln	
45 50 55	
GCC GTA GAA GTC TGG CAG GGC CTG GCC CTG CTG TCG GAA GCT GTC CTG	241
Ala Val Glu Val Trp Gln Gly Leu Ala Leu Ser Glu Ala Val Leu	
60 65 70 75	

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CGG GGC CAG GCC CTG TTG GTC AAC TCG AGC CAG CCG TGG GAG CCC CTG	289
Arg Gly Gln Ala Leu Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu	
80 85 90	
CAA CTG CAT GTG GAT AAA GCC GTC AGT GGC CTT CGC AGC CTC ACC ACT	337
Gln Leu His Val Asp Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr	
95 100 105	
CTG CTT CGG GCT CTG GGA GCT CAG AAG GAA GCC ATC TCC CCT CCA GAT	385
Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp	
110 115 120	
GCG GCC TCA GCT GCT CCA CTC CGA ACA ATC ACT GCT GAC ACT TTC CGC	433
Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg	
125 130 135	
AAA CTC TTC CGA GTC TAC TCC AAT TTC CTC CGG GGA AAG CTG AAG CTG	481
Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu	
140 145 150 155	
TAC ACA GGG GAG GCA TGC AGG ACA GGG GAC AG ATGATAAGGA TCCGAATT	532
Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp	
160 165	

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 556 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 14..538

(ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 17..538

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AAGCTTACCT GCC ATG ACC CCC CTG GGC CCT GCC AGC TCC CTG CCC CAG	49
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln	
-1 1 5 10	

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AGC TTC CTG CTC AAG TGC TTA GAG CAA GTG AGG AAG ATC CAG GGC GAT	97		
Ser Phe Leu Leu Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp			
15	20	25	
GGC GCA GCG CTC CAG GAG AAG CTG TGT GCC ACC TAC AAG CTG TGC CAC	145		
Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His			
30	35	40	
CCC GAG GAG CTG GTG CTG CTC GGA CAC TCT CTG GGC ATC CCC TGG GCT	193		
Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala			
45	50	55	
CCC CTG AGC TCC TGC CCC AGC CAG GCC CTG CAG CTG GCA GGC TGC TTG	241		
Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu			
60	65	70	75
AGC CAA CTC CAT AGC GGC CTT TTC CTC TAC CAG GGG CTC CTG CAG GCC	289		
Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala			
80	85	90	
CTG GAA GGG ATA TCC CCC GAG TTG GGT CCC ACC TTG CAC ACA CTG CAG	337		
Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu His Thr Leu Gln			
95	100	105	
CTG GAC GTC GCC GAC TTT GCC ACC ACC ATC TGG CAG CAG ATG GAA GAA	385		
Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu			
110	115	120	
CTG GGA ATG GCC CCT GCC CTG CAG CCC ACC CAG GGT GCC ATG CCG GCC	433		
Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala			
125	130	135	
TTC GCC TCT GCT TTC CAG CGC CGG GCA GGA GGG GTC CTG GTT GCT AGC	481		
Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser			
140	145	150	155
CAT CTG CAG AGC TTC CTG GAG GTG TCG TAC CGC GTT CTA CGC CAC CTT	529		
His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu			
160	165	170	
GCG CAG CCC TGATAAGGAT CCGAATTG	556		
Ala Gln Pro			

## (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 39 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AATTGCCGCC ACCATGAGCC GCCTGCCCGT CCTGCTCCT

39

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCTCCAACTC CTGGTCCGCC CCGGACTCCA AGCTCCCATG ACCCAGACAA

50

(2) INFORMATION FOR SEQ ID NO:21.

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTAGTTGTCT GGGTCATGGG AGCTTGGAGT CCGGGGGCGG

39

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(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 50 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ACCAAGGAGTT GGAGCAGGAG CAGGACGGGC AGGCAGGCTCA TGGTGGCGGC 50

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTAGCGATCT TTCTAGA 17

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CATGTCTAGA AAGATCG

17

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 57 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CTAGCGTCCG GAGGCAGGTGG CTCGGGCGGT GGCAGGCTCGG GTGGCGGCAG CTCTGCG

57

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 57 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTAGCGCAGA GCCGCCGCCA CCGCAGCCGC CACCGCCCCA GCCACCGCCT CCGGACG

57

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 amino acids

- 60 -

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TTGTCGCTAG CGTCCGGAGG C

21

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CTAGAACCGG CCGCA

15

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 15 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TTCGCCGGCG TTCGA

15

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TCGAGCCATG GGGGTGCACG AATGTCTT

28

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

- 62 -

- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GCCTGGCTGT GGCTTCTCCT GTCCCTGCTG TC

32

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 32 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GCTCCCTCTG GGCCTCCCAG TCCTGGGCTG CA

32

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GCCCAGGACT GGGAGGCCA GAGGGA

26

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(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCGACAGCAG GGACAGGAGA AGCCACAGCC AGGCAGGACA TT

42

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 16 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CGTGCACCCC CATGGC

16

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GCCCCACCAAC GCCTCATCTG T

21

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GAATTCGGAT CCTTATCATC T

21

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CTAGTCTCTA GAATGGGGGT CCACGAATGT

30

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

AGCCATGGCG GCCGCTCTGT CCCCTGTCCT

30

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GACAGAGCGG CCGCCATGGC TCCCATGACC

30

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GAATTCTGGAT CCTTACTAAA AGATCGCTAG

30

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(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 57 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GGCCGCTTCC GGAGGCAGGTG GCTCGGGCGG TGGCGGCTCG GGTGGCGGCG GCTCTGC

57

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 57 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GGCCGCAGAG CCGCCGCCAC CCGAGCCGCC ACCGCCGAG CCACCGCCTC CGGCAGC

57

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What is claimed is:

1. A recombinant hematopoietic molecule comprising at least a portion of a first hematopoietic molecule having early myeloid differentiation activity and at least a portion of a second hematopoietic molecule having late myeloid differentiation activity, said recombinant hematopoietic molecule having early myeloid differentiation activity associated with said first hematopoietic molecule and late myeloid differentiation activity associated with said second hematopoietic molecule.
2. A recombinant hematopoietic molecule of claim 1 wherein the first hematopoietic molecule is selected from the group consisting of IL-3 and GM-CSF.
3. A recombinant hematopoietic molecule of claim 1 wherein the second hematopoietic molecule is selected from the group consisting of Epo, G-CSF, IL-5 and M-CSF.
4. A recombinant hematopoietic molecule of claim 1 wherein the portion of the first hematopoietic molecule is linked to the portion of the second hematopoietic molecule by an amino acid linker sequence of at least two amino acid residues.
5. A recombinant hematopoietic molecule of claim 1 comprising SEQ ID NO: 1.
6. A recombinant hematopoietic molecule of claim 1 comprising an amino acid sequence contained within SEQ ID NO: 2.
7. A recombinant hematopoietic molecule of claim 1 comprising SEQ ID NO: 3.
8. A recombinant hematopoietic molecule of claim 1 comprising an amino acid sequence contained within SEQ ID NO: 4.

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9. A recombinant hematopoietic molecule of claim 1 comprising SEQ ID NO: 5.

5 10. A recombinant hematopoietic molecule of claim 1 wherein the first hematopoietic molecule is IL-3 and the second hematopoietic molecule is Epo.

10 11. A recombinant hematopoietic molecule of claim 10 wherein the first hematopoietic molecule comprises the amino portion and the second hematopoietic molecule comprises the carboxy portion of the recombinant hematopoietic molecule.

15 12. A recombinant hematopoietic molecule of claim 11 which comprises SEQ ID NO: 6.

13. A recombinant hematopoietic molecule of claim 11 which comprises SEQ ID NO: 7.

20 14. A recombinant hematopoietic molecule of claim 10 wherein the first hematopoietic molecule comprises the carboxy portion and the second hematopoietic molecule comprises the amino portion of the recombinant hematopoietic molecule.

25 15. A recombinant hematopoietic molecule of claim 14 which comprises SEQ ID NO: 8.

16. A recombinant hematopoietic molecule of claim 14 which comprises SEQ ID NO: 9.

30 17. A recombinant hematopoietic molecule of claim 1 wherein the first hematopoietic molecule is IL-3 and the second hematopoietic molecule is G-CSF

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18. A recombinant hematopoietic molecule of claim 17 wherein the first hematopoietic molecule comprises the amino portion and the second hematopoietic molecule comprises the carboxy portion of the recombinant hematopoietic molecule.

5

19. A recombinant hematopoietic molecule of claim 18 which comprises SEQ ID NO: 10.

10 20. A nucleic acid molecule which encodes the recombinant hematopoietic molecule of claim 1.

21. An expression vector which comprises the nucleic acid molecule of claim 20.

15 22. A host cell transformed with the expression vector of claim 19.

23. A host cell of claim 22 which comprises a mammalian cell.

20

24. A method for producing a recombinant hematopoietic molecule comprising at least a portion of a first hematopoietic molecule having early myeloid differentiation activity and at least a portion of a second hematopoietic molecule having late myeloid differentiation activity, which comprises culturing a host cell of claim 22 under suitable conditions so as to allow the expression of such recombinant hematopoietic molecule, and recovering such recombinant hematopoietic molecule.

30

25. A pharmaceutical composition which comprises a recombinant hematopoietic molecule of claim 1 and a pharmaceutically acceptable carrier.

35

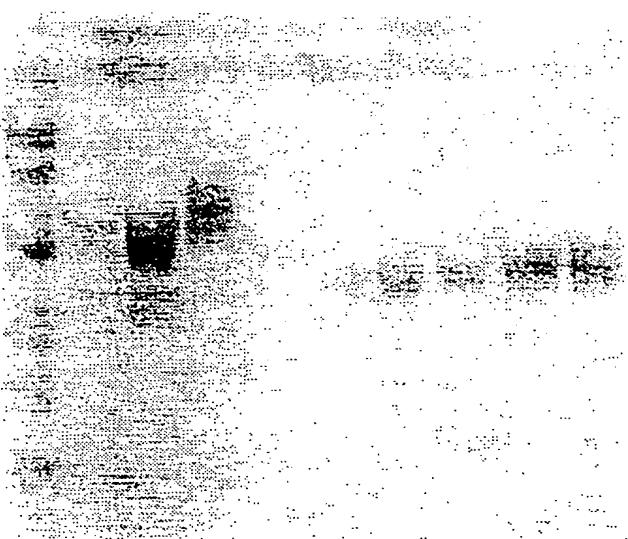
26. A method for promoting hematopoiesis in a patient which comprises administering to such patient a pharmaceutical composition of claim 25.

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# FIG-1

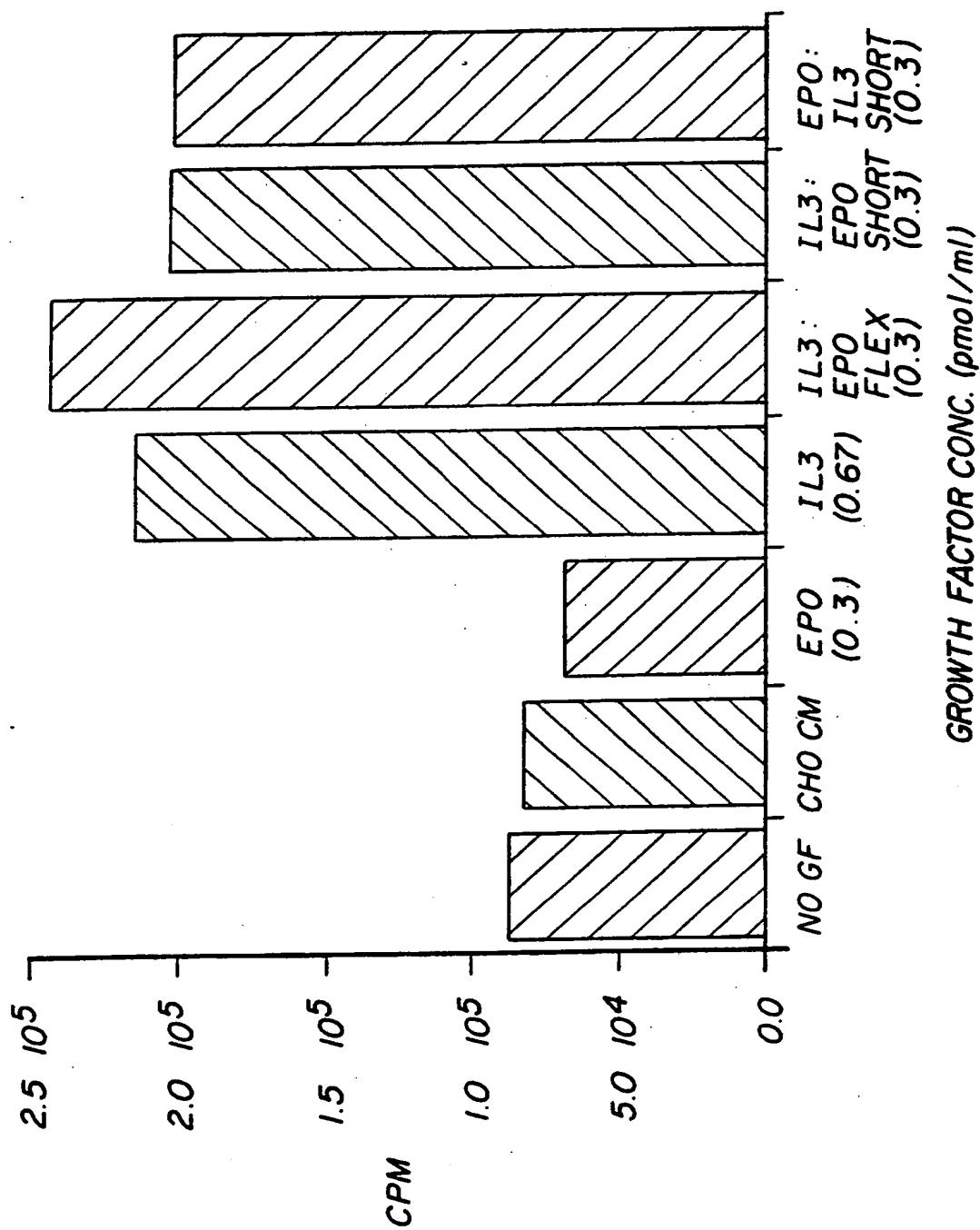
1. 2. 3. 4. 5. 6. 7. 8. 9. 10.

106 —  
80 —  
49.5 —  
32.5 —  
27.5 —  
18.5 —



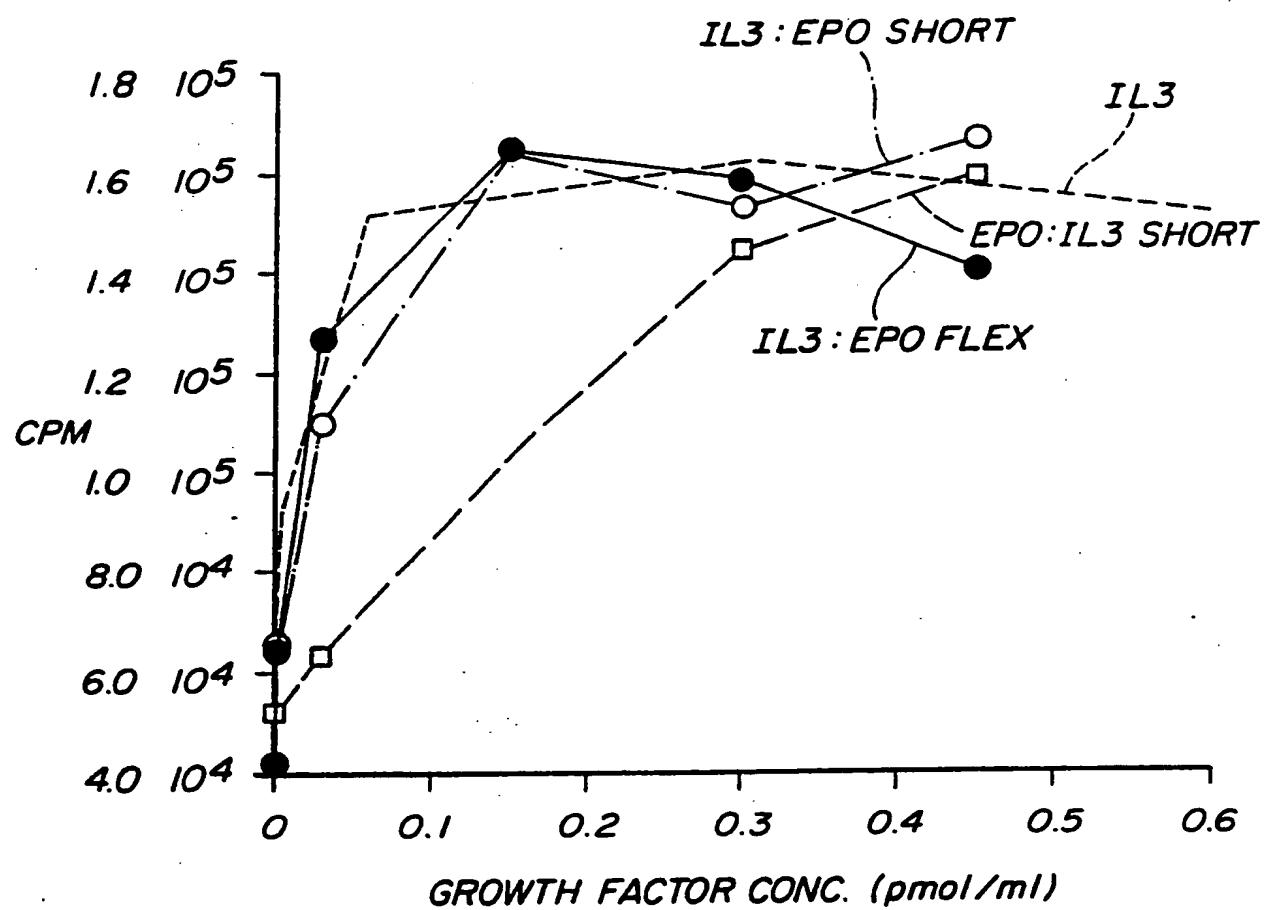
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FIG-2



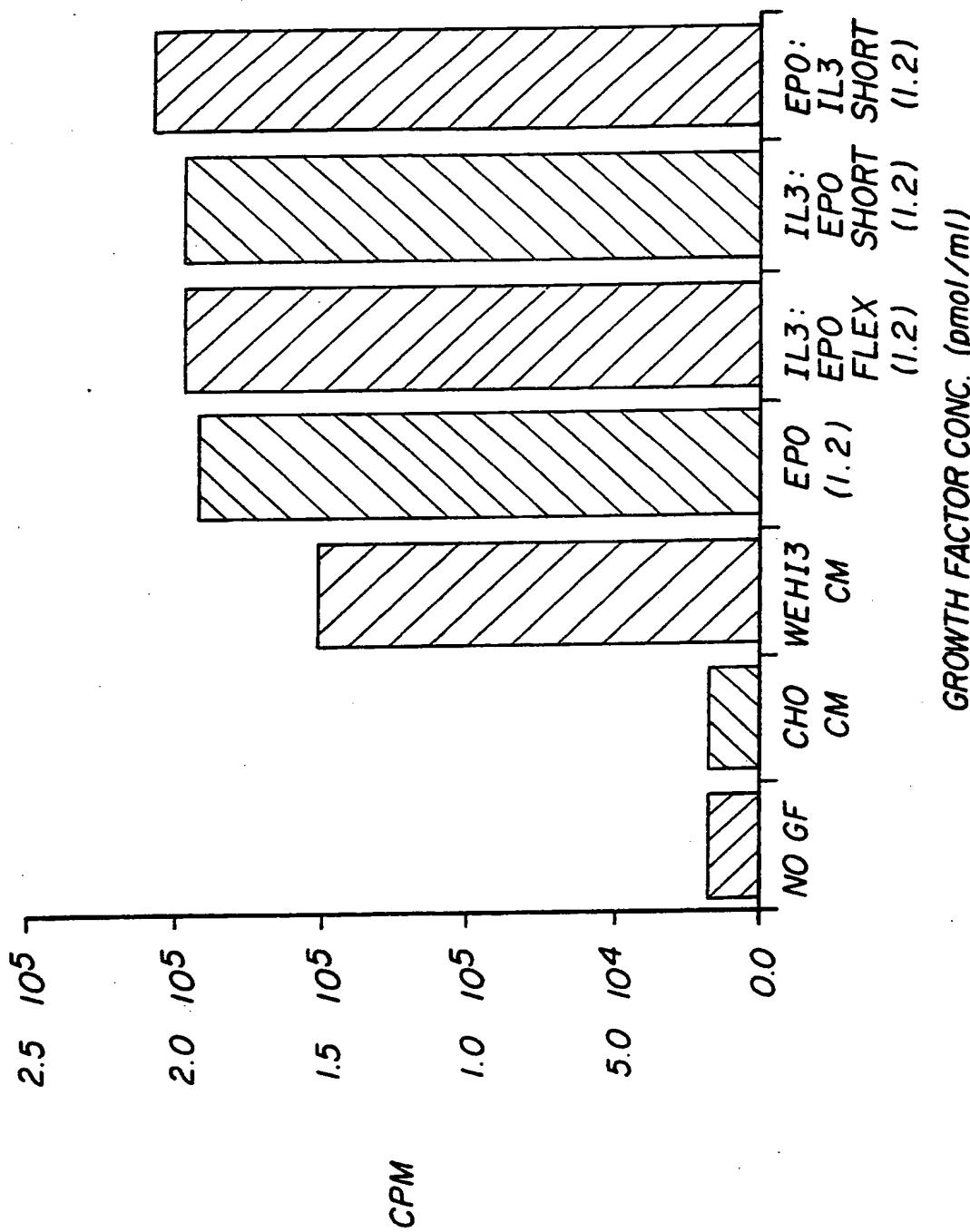
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FIG-3



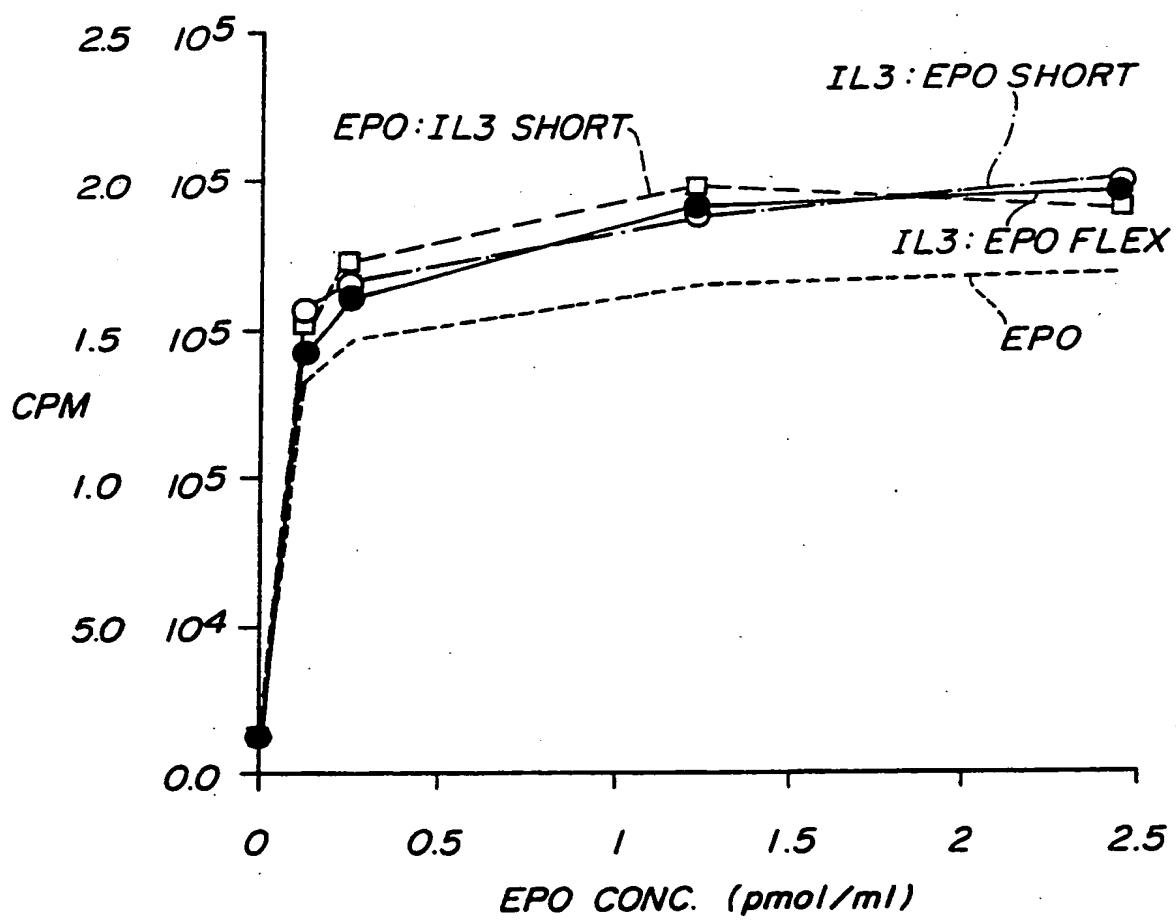
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FIG-4



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FIG-5



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FIG-6

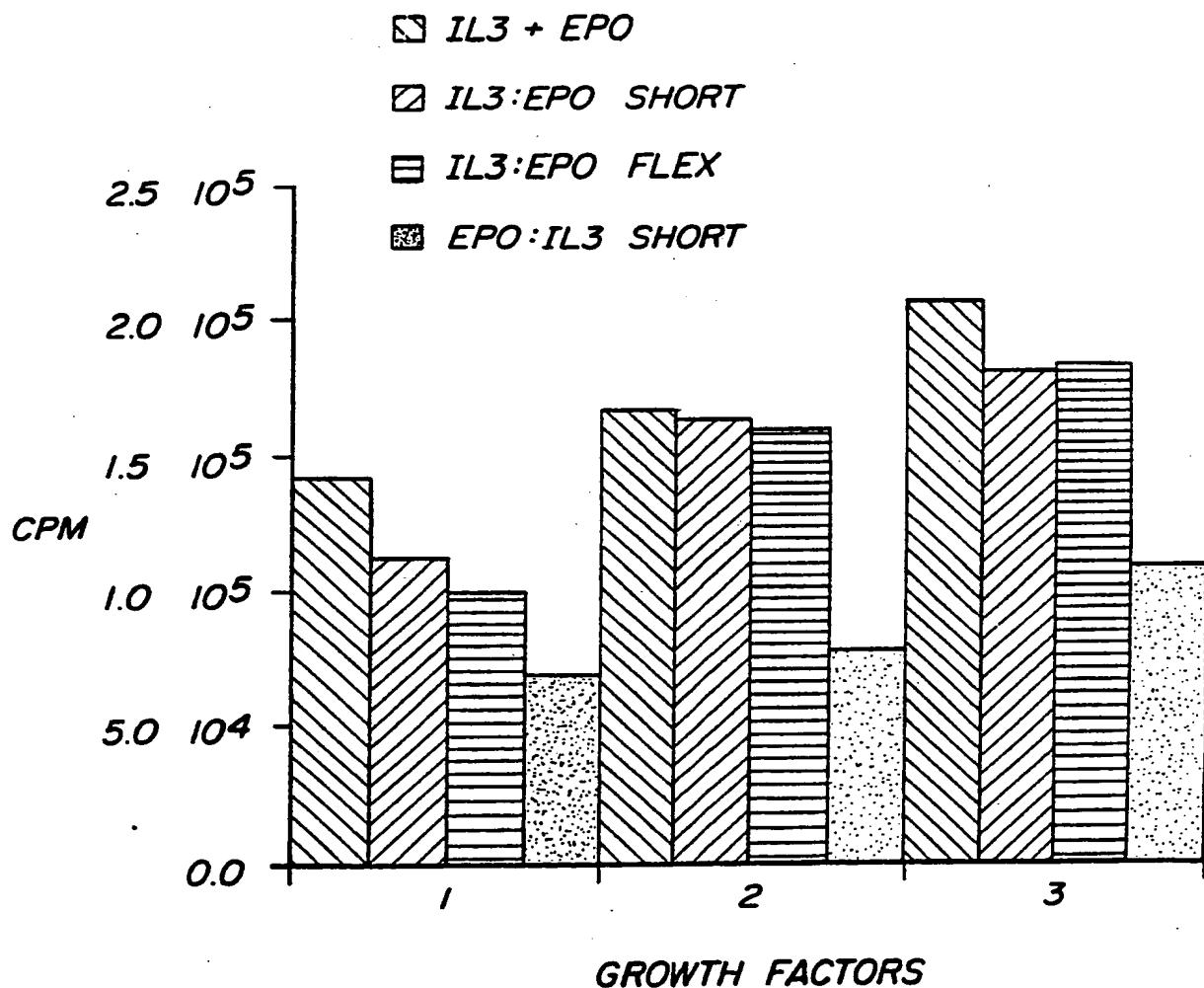


FIG-7A

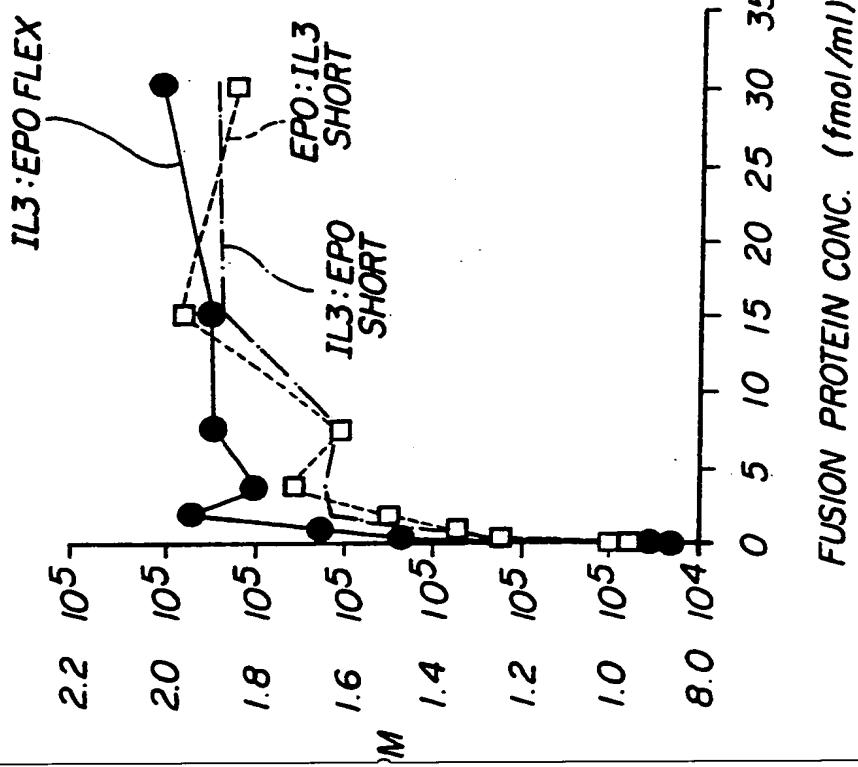
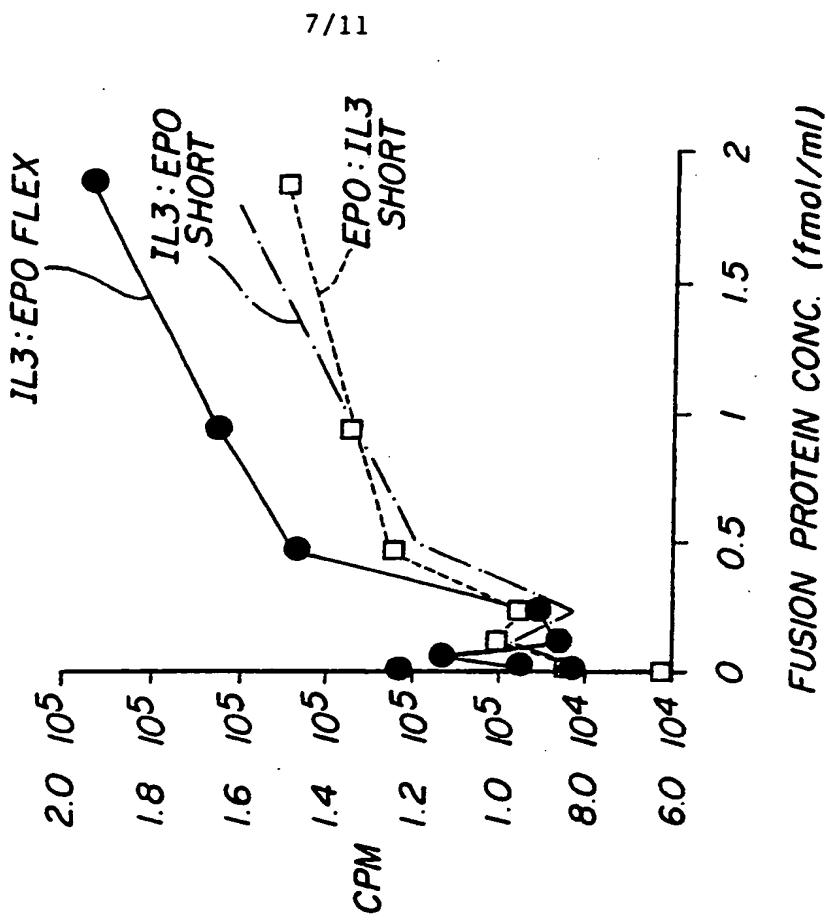


FIG-7B



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FIG-8A

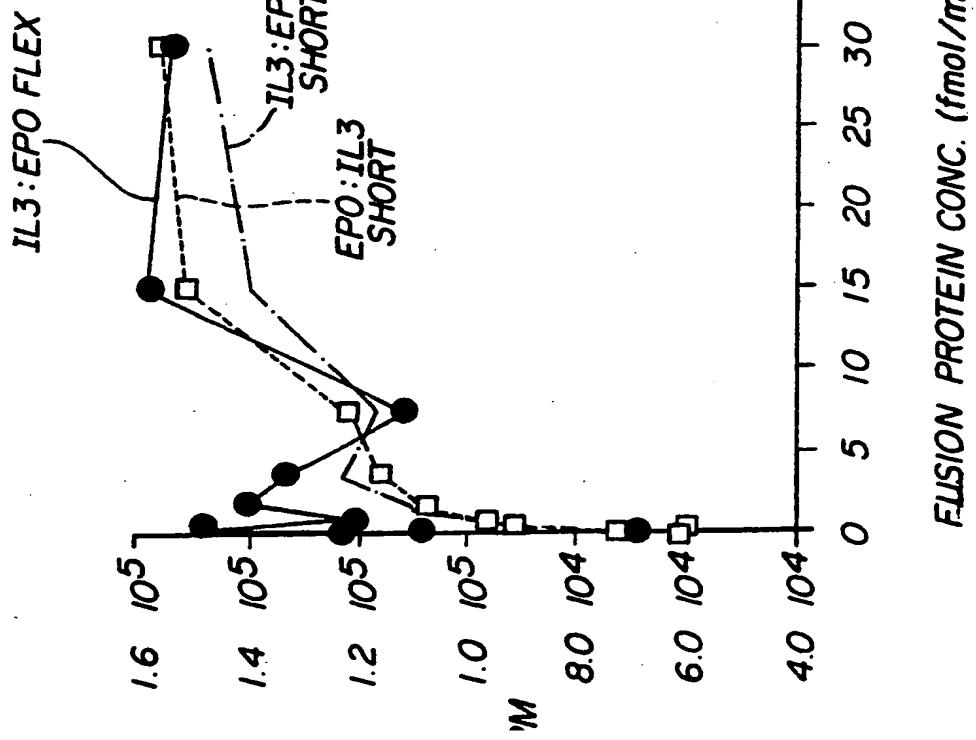
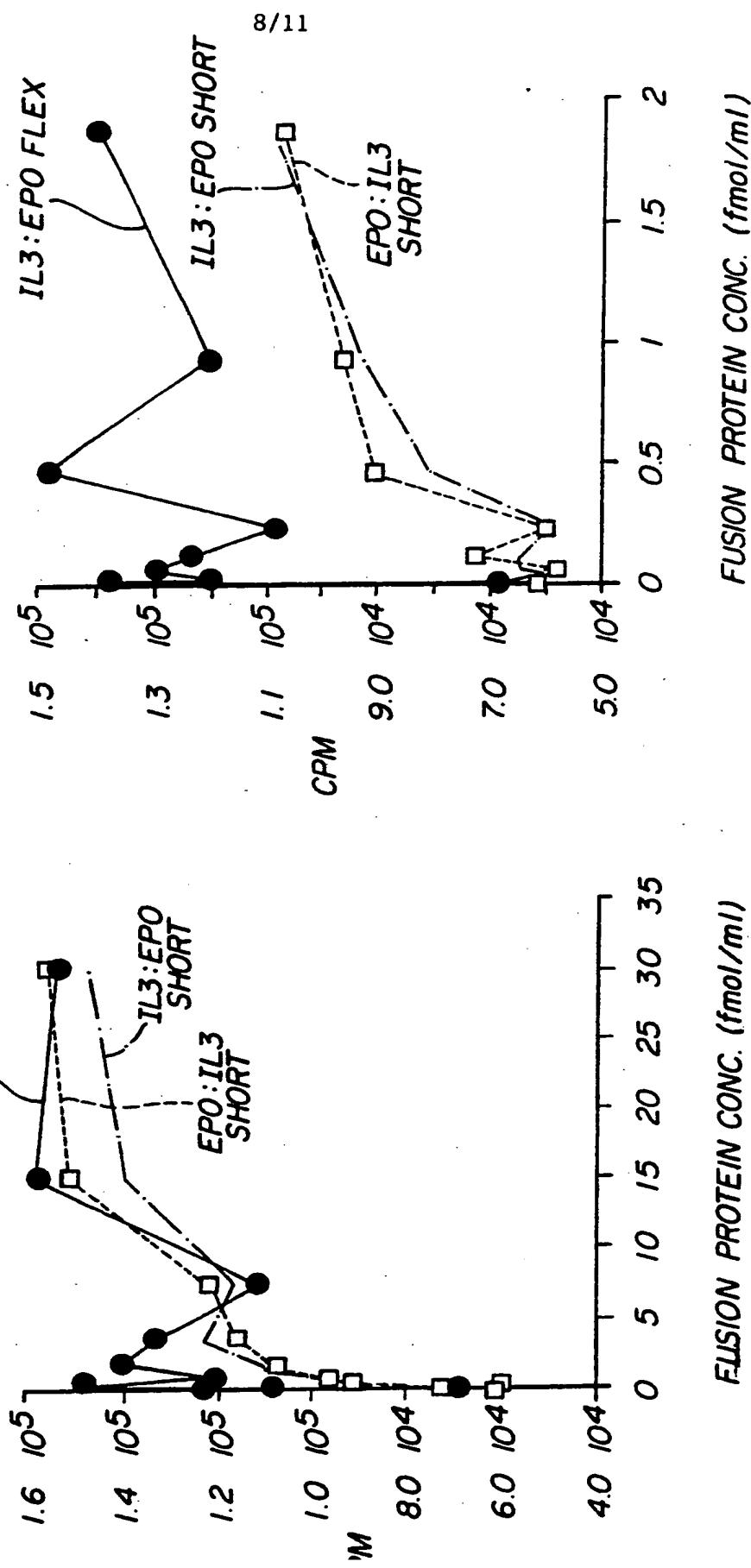


FIG-8B



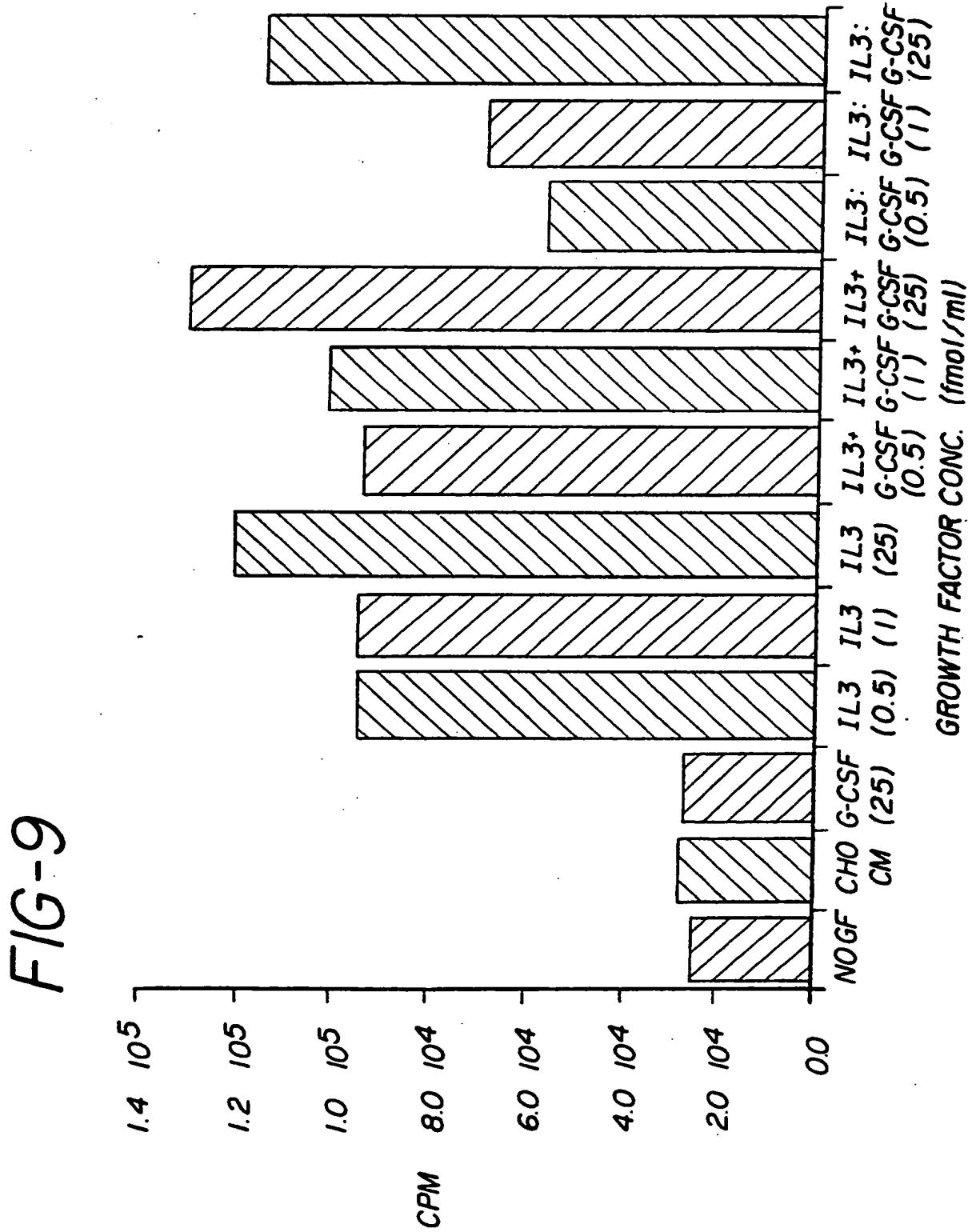
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FUSION PROTEIN CONC. (fmol/ml)

FUSION PROTEIN CONC. (fmol/ml)

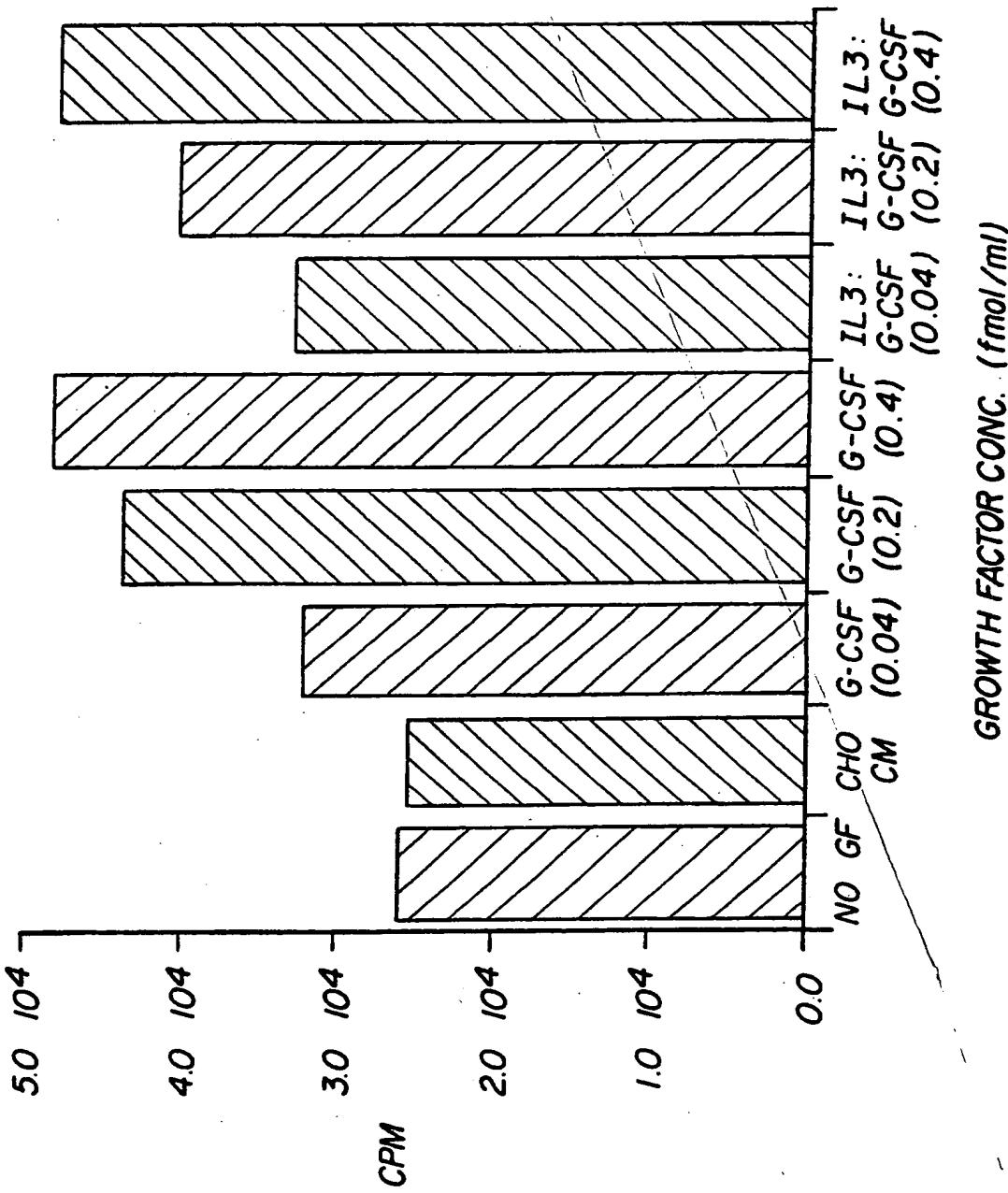
FUSION PROTEIN CONC. (fmol/ml)

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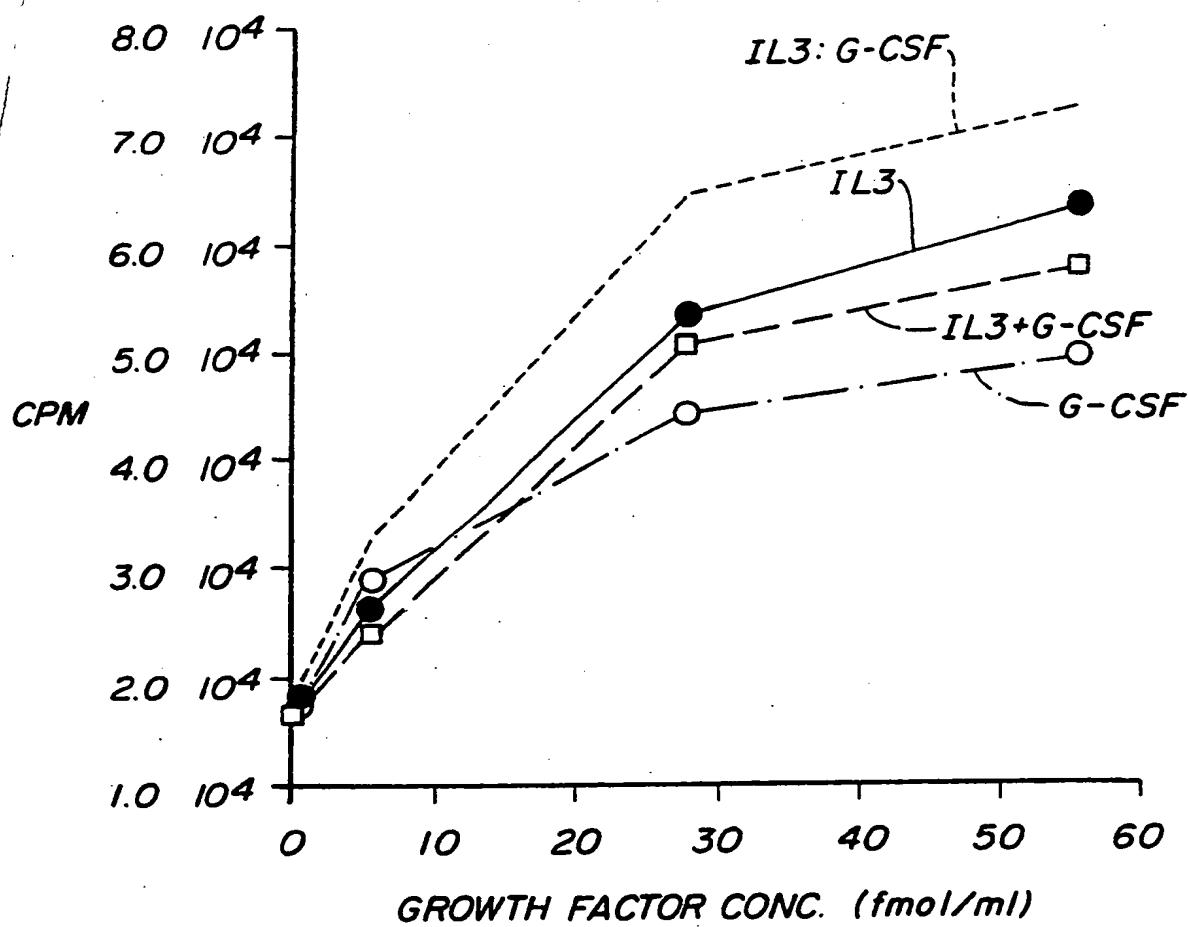
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FIG-10



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FIG-11



# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/07053

## I. CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all) <sup>6</sup>

According to International Patent Classification (IPC) or to both National Classification and IPC  
 IPC(5):C07K 15/00; C12P21/02; C12N 15/24, 15/27, 15/70, 5/00,5/10; A61K 37/02; C07H 15/12  
 U.S. CL.: 530/351,395;435/69.5,69.51,69.6,69.7, 172.3,240.1,320.1;424/85.1; 536/27

## II. FIELDS SEARCHED

Minimum Documentation Searched <sup>7</sup>

Classification System	Classification Symbols
U.S.	530/350,351,395; 435/69.1,69.5,69.51, 69.52, 69.6,69.7,172.3 240.1,252.3, 320.1; 424/85.1,85.2; 536/27

Documentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>

Computer Data-base search

## III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup>

Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
E, X Y	U.S. A. 5.073.627 (Curtis et al.) 17 December 1991. see claims.	1-2,20-26,4 1-26
Y	U.S. A. 4.935.233 (Bell et al.) 19 June 1990. see col. 3-4 and claims.	1-26
Y	U.S. A. 4.935.352 (Koichi et al.) 19 June 1990. see all.	1-26
P,Y	WO. A. 91/01004 (Svrluga et al.) 24 January 1991. see all.	1-26
A	U.S. A. 4.675.382 (Murphy) 23 June 1987. see all.	1-26
Y	WO. A. 88/00971 (Ramshaw et al.) 11 February 1988. see all.	1-26

\* Special categories of cited documents: <sup>10</sup>

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

## IV. CERTIFICATE

Date of the Actual Completion of the International Search

09 January 1992

Date of Mailing of this International Search Report

07 FEB 1992

International Searching Authority

ISA/US

Signature of Authorized Officer

Garnette D. Draper

*Garnette D. Draper*  
ebw

